Studies on Ansamycin Antibiotics:

Mutasynthetic Approach Towards New Rifamycin Derivatives

and

Total Synthesis of Progeldanamycin Derivatives

Von der Naturwissenschaftlichen Fakultät
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von
Ilona Bułyszko, magister (Polen)
geboren am 12.10.1987 in Góra, Polen

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Hierdurch erkläre ich, dass die vorliegende Dissertation selbständig verfasst und alle benutzten Hilfsmittel sowie eventuell zur Hilfeleistung herangezogenen Institutionen vollständig angegeben wurden.

Die Dissertation wurde nicht schon als Master- oder ähnliche Prüfungsarbeit verwendet.

Hannover, den 19.09.2016

Referent: Prof. Dr. Andreas Kirschning

Korreferent: Prof. Dr. Markus Kalesse

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To my grandmother

(1935-2016)

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Zussamenfassung

Ilona Bułyszko

Studien zu Ansamycin Antibiotika: Mutasynthetischer Ansatz zu neuen Rifamycin-Derivaten und Totalsynthese von Progeldanamycin-Derivaten

Schlagworte: Rifamycin, Geldanamycin, Totalsynthese, Mutasynthese, Polyketidsynthase

Die Ansamycin Antibiotika sind Makrolactampolyketide, bestehend aus einer aliphatischen Brücke (*lat. ansa* = Henkel), welche in zwei nicht-benachbarten Positionen Benzyl- und Naphthylgruppen enthalten. Naphthalen enthaltene Ansamycine (z. B. Rifamycin) weisen überwiegend antimikrobielle Aktivitäten auf, während Benzol enthaltene Ansamycine (z. B. Geldanamycin) antikanzerogene Eigenschaften besitzen. Beide Vertreter der Ansamycin Antibiotika, Rifamycin und Geldanamycin, werden über eine Typ I Polyketidsynthase (PKS), ausgehend von 3-Amino-5-hydroxybenzoesäure (AHBA), biosynthetisiert.

Im ersten Teil dieser Doktorarbeit wurde, unter Verwendung des Konzepts der Mutasynthese, das synthetische Potential der (AHBA)-Blockmutante *A. mediterranei* HGF003, welche Rifamycin B produziert, untersucht. Dreißig, verschieden substituierte und funktionalisierte Benzoesäuren, wurden der Blockmutante supplementiert, wobei dreizehn Tetraketide gebildet und isoliert wurden. Obwohl die isolierten 2*H*-Pyran-2-on-Derivate keine biologischen Aktivitäten aufweisen, können einige von ihnen als chirale Bausteine für die Synthese von komplexeren Intermediaten verwendet werden. Die Resultate offenbaren eine hohe Toleranz der Rifamycin Ladungsdomäne für unnatürliche Benzoesäuren und eine hohe Substratspezifität der bei der Naphthalenbildung beteiligten Enzyme. Zusätzlich enthüllen die Fütterungsexperimente mit Azid- und Nitroanaloga von AHBA, die einzigartigen reduktiven Eigenschaften von *A. mediterranei* bezogen auf Arylazide und Nitroarene. Dies ist bisher erst die zweite literaturbekannte mikrobielle Quelle, welche reduktive Eigenschaften besitzt.

Im zweiten Teil dieser Arbeit wurden Untersuchungen zur Synthese von vier *seco*-Progeldanamycinsäure-Derivaten vorgestellt, von denen ein *seco*-Progeldanamycin erfolgreich dargestellt werden konnte. Dieses Derivat wird als Substrat für Studien zur Flexibilität der Amidsynthase (GdmF), einem Schlüsselenzym in der Geldanamycin Biosynthese, welcher für die Makrolactamisierung zuständig ist, benutzt werden, um die Toleranz und die biosynthetische Umwandlung des Enzyms zu untersuchen. Zusätzlich konnten auch die SNAc-Ester zu einem *Knockout* Stamm von *S. hygroscopicus var. geldanus* (K-390-61-1) supplementiert werden, um neue Geldanamycin-Derivate zu erhalten.

Abstract

Ilona Bułyszko

Studies on Ansamycin Antibiotics: Mutasynthetic Approach Towards New Rifamycin Derivatives and Total Synthesis of Progeldanamycin Derivatives

Keywords: rifamycin, geldanamycin, total synthesis, mutasynthesis, polyketide synthase

The ansamycin antibiotics are macrolactam polyketides consisting of an aliphatic bridge linking two non-adjacent positions of a benzenic or naphthalenic moiety (from the Latin *ansa* means a handle). Naphthalenic ansamycins (e.g. rifamycins) exhibit mainly antimicrobial activities, while benzenic ansamycins (e.g. geldanamycin) display anticancer properties. Both representatives of the ansamycin antibiotics, rifamycin and geldanamycin, are synthesized by type I polyketide synthase (PKS) assembled from 3-amino-5-hydroxybenzoic acid (AHBA).

In the first part of this doctoral thesis the synthetic potential of the AHBA(−) mutant strain of *A. mediterranei* HGF003, the producer of rifamycin B, has been evaluated using the concept of mutasynthesis. Thirty differently substituted and functionalized benzoic acids were fed to the mutant strain resulting in the formation and the isolation of thirteen tetraketides. Although the isolated 2*H*-pyran-2-one derivatives do not display any biological activity, some of them can be employed as chiral building blocks in the synthesis of more advanced intermediates. These results revealed high tolerance of the rifamycin loading domain to unnatural benzoic acids and high substrate specificity of the enzymes involved in naphthalene formation. Additionally, feeding experiments with azido- and nitro analogues of AHBA unveiled unique reductive properties of *A. mediterranei* towards aryl azides and nitroarenes. It has been the second microbial source possessing reductive properties towards azidoarenes reported so far in the literature.

In the second part of this work the synthesis of four *seco*-progeldanamycin acid derivatives was studied, out of which one *seco*-progeldanamycin acid derivative has been successfully synthesized. It will be used as a substrate in the studies on the flexibility of the amide synthase (GdmF), a key enzyme in the geldanamycin biosynthesis responsible for the macrolactamization, to investigate the tolerance and biosynthetic transformation by the enzyme. Additionally, the prepared SNAc ester could be fed to a knockout strain of *S. hygroscopicus var. geldanus* (K-390-61-1) to obtain new geldanamycin derivative.

Contents

1 Rifamycins

1.1 Introduction

The ansamycins comprise a class of macrolactam polyketides which exhibit a broad spectrum of antimicrobial activity against Gram-positive and some Gram-negative bacteria and are used as antibiotics, anticancer agents and enzyme inhibitors.[1] The name of ansamycins has an origin in the latin word *ansa* which means "a handle" – in this case represented by an aliphatic bridge linking two non-adjacent positions of a benzenic or naphthalenic moiety (Figure 1).[2–4] Naphthalenic ansamycins (rifamycins, streptovaricins, naphthomycins) display mainly antimicrobial activities, while benzenic ansamycins (geldanamycin, ansamitocin, ansatrienine) demonstrate anticancer properties.[1,4]

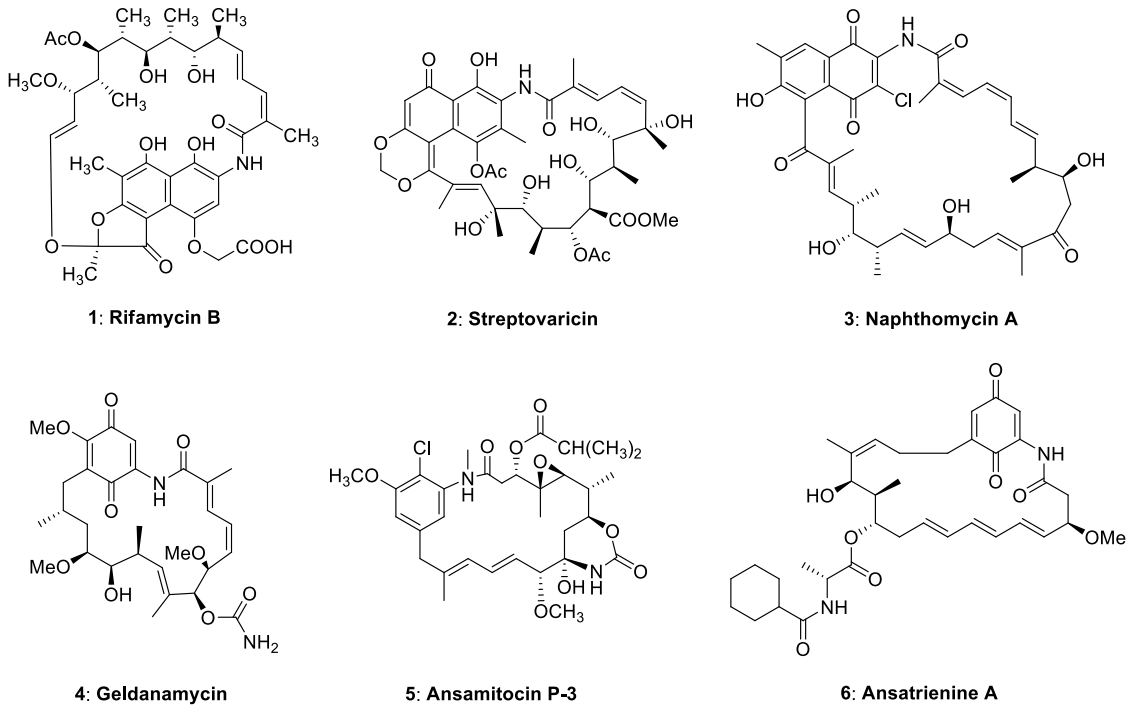
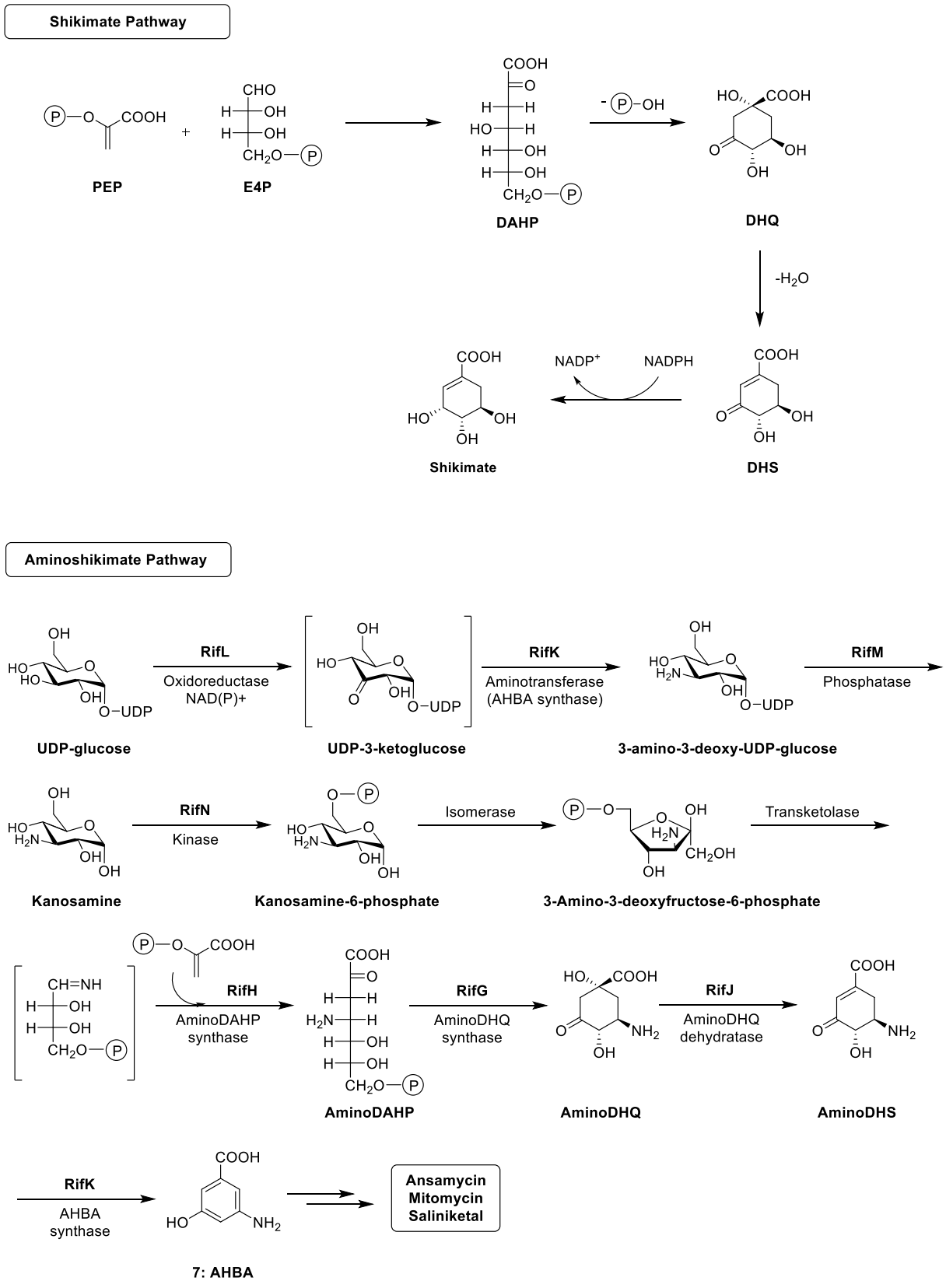


Figure 1. Structures of ansamycin antibiotics.

A structural component typical of all ansamycin antibiotics is the so-called mC_7N unit consisting of a six-membered carbocycle, usually aromatic or quinoid, bearing extra carbon and nitrogen atom in a *meta* arrangement.[3,5–7] Studies on the biosynthesis of rifamycins carried out independently by two research groups in 1981 revealed 3-amino-5-hydroxybenzoic acid (AHBA, **7**) as a specific precursor of the mC_7N unit.



Scheme 1. The shikimate pathway and the aminoshikimate pathway of AHBA formation.[3]

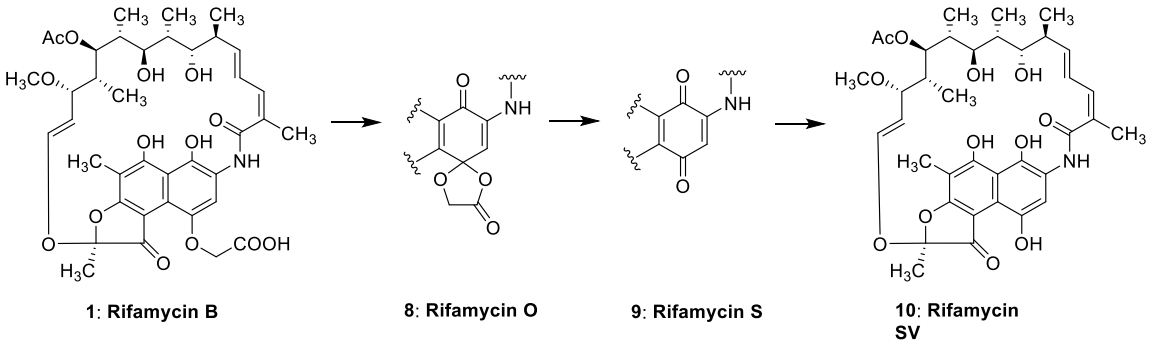
In 1981 Ghisalba and Nüesch published the results of the feeding experiments with the use of a mutant of *Amycolatopsis mediterranei* blocked in an early step of the rifamycin biosynthesis.[8] They found that supplementation with the compound **7** restored rifamycin production in the mutant strain. In the same year the Rickards group performed feeding experiments with ^{14}C and ^{13}C-labeled AHBA which showed that compound **7** was efficiently incorporated into the mC_7N unit of actamycin, rifamycin, ansamitocin and mitomycin.[9–11] In the following years the incorporation of AHBA into the benzenic ansamycins was reported.[12–14] However, all the published results did not explain the formation of 3-amino-5-hydroxybenzoic acid in the shikimate pathway (Scheme 1). The key question was whether the nitrogen atom present in AHBA was attached to C-3 or C-5 of the cyclic shikimate pathway intermediates. According to the work of Hornemann and co-workers,[15] the origin of the AHBA related to the aminoshikimate pathway was proposed. Erythrose-4-phosphate (E4P) was suggested to react with the ammonia generated *in situ* by hydrolysis of glutamine, to form an imine which is condensed with phosphoenolpyruvate (PEP) to give aminoDAHP - a key intermediate in the synthesis of AHBA. Cyclization to aminoDHQ and the following dehydration were proposed to provide aminoDHS which is aromatized to AHBA. The aminoshikimate pathway was proven by the preparation of the three postulated intermediates which were used for incubation with cell-free extracts of the rifamycin producer − *A. mediterranei*. The observed conversion of all of these compounds to AHBA (aminoDAHP 45%, aminoDHQ 41%, aminoDHS 100%) and no conversion of DAHP supported the hypothetical aminoshikimate pathway.[5]

The complete pathway of AHBA formation was published by Floss and co-workers in 2011 (Scheme 1).[3] Studies on the gene clusters of AHBA synthesis revealed that kanosamine is a nitrogenous precursor. This compound is then phosphorylated and converted by a set of enzymes into 1-deoxy-1-imino-erythrose-4-phosphate – a substrate for the aminoDAHP formation. The further steps from the stage of aminoDAHP to AHBA in the aminoshikimate pathway were validated.

1.1.1 General Information about Rifamycins

The rifamycins belong to the group of naphthalenic ansamycins and were first isolated in 1957 from a fermentation culture of *Amycolatopsis mediterranei* (previously classified as *Streptomyces mediterranei* or *Nocardia mediterranei*) by Sensi and co-workers at the laboratory of Gruppo Lepetit in Milan, Italy, as a mixture of several congeners. In 1973 White

and co-workers established the biosynthetic origin of rifamycin S and proposed a general scheme for the ansamycin biogenesis based on NMR studies with ^{13}C-enriched precursors.[16]



Scheme 2. Conversion of rifamycin B to rifamycin SV and examples of clinically used rifamycin derivatives.[2]

The rifamycins display antimicrobial activity against Gram-positive bacteria, particularly mycobacteria and some Gram-negative bacteria. Rifamycin B (**1**) which is the main product of the commercial fermentation and exhibits only slight activity can be converted chemically, enzymatically or by biotransformation to the active rifamycin SV (**10**, Scheme 2), the first clinically used rifamycin.

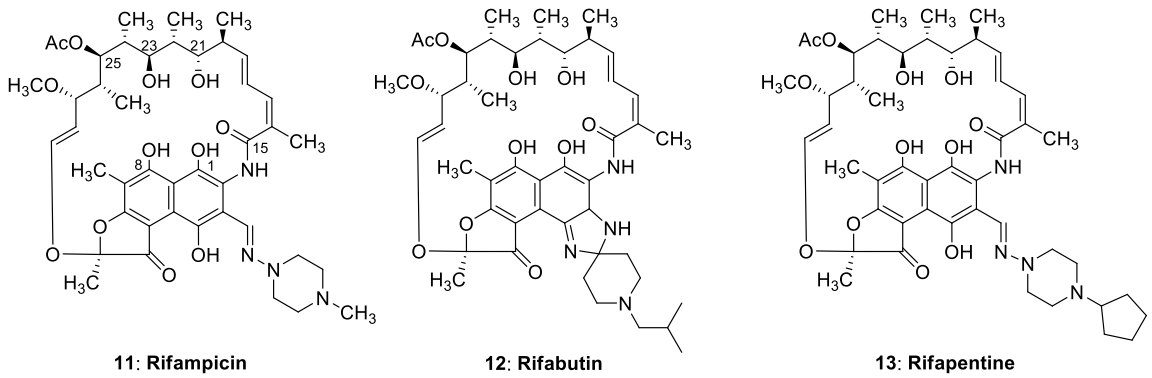


Figure 2. Clinically used rifamycin derivatives.

Another compound with enhanced activity against Gram-positive bacteria is rifampicin (**11**) almost always used along with other antibiotics. It displays good activity also against Gram-negative bacteria and excellent oral bioavailability. Deemed one of the most in-demand medications nowadays, rifampicin is used in the treatment of tuberculosis, leprosy, Legionnaire's disease and AIDS-associated microbacterial infections called *Mycobacterium avium complex* (MAC).[17] The antibiotic **11** is also known for its ability to inhibit multidrug resistance by enhancing the anticancer drug's accumulation in cancer cells.[18,19] Following

the clinical introduction of rifamycin SV and rifampicin other rifamycin derivatives: rifabutine (**12**) and rifapentine (**13**, Figure 2) were successfully tested and brought to the market.[2]

1.1.2 Mechanism of Action

The rifamycins display their activity through inhibition of DNA-dependent RNA polymerases of prokaryotes. Simultaneously, they exhibit very low activity against eukaryotic RNA polymerases which renders them effective and safe drugs. This mechanism was found to be common for all antimicrobially active rifamycins.[2]

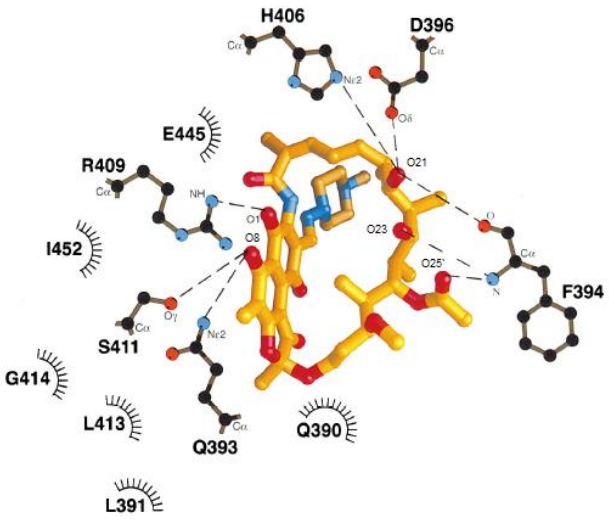


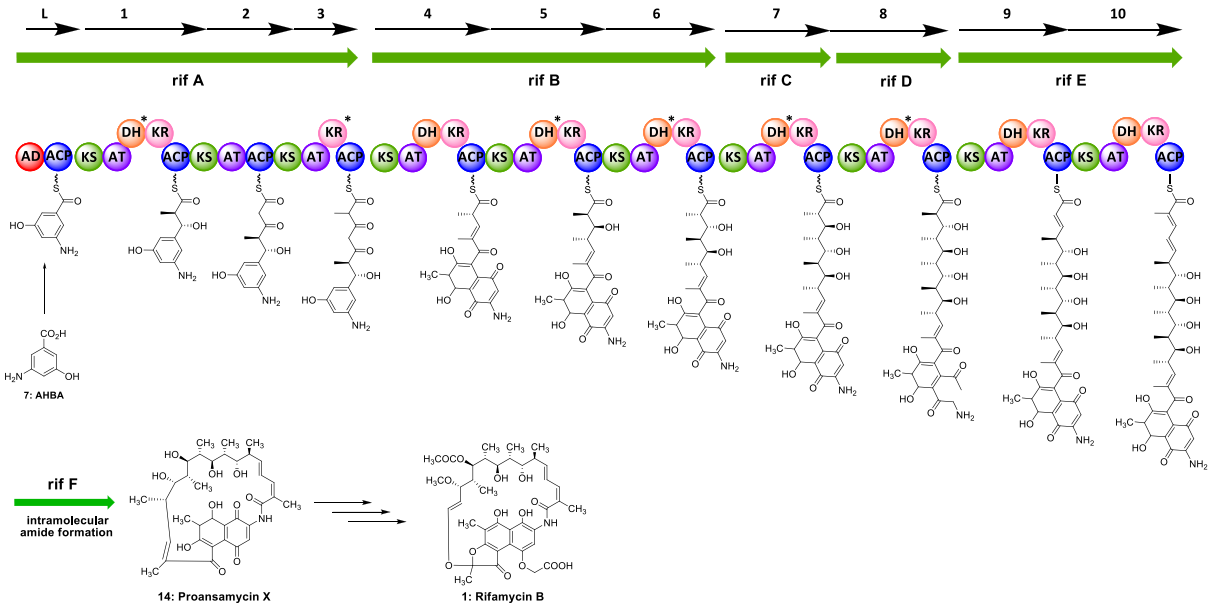
Figure 3. Interaction of rifampicin with amino acids of *Thermus aquaticus* DNA-dependent RNA polymerase (modified from the ref.[20]).

The DNA-dependent RNA polymerase is a complex enzyme producing primary transcript RNA and constists of five subunits: α^{I}, α^{II}, β, β' and ω. Studies on the interaction of the enzyme from *Thermus aquaticus* with rifampicin (**11**) provided a detailed functional model for rifampicin's mode of action.[20,21] It validated the earlier work done by Hartmann and Nitta who observed that rifampicin acts only in an early stage of the RNA synthesis by a physical interruption of the growing oligonucleotide chain after the first or the second elongation step.[22,23] The binding site for rifampicin has been found at the subunit β which is encoded by the *rpo*B gene. The connection to the enzyme involves hydrogen bonding interactions between the four hydroxyl groups (at C-1, C-8, C-21 and C-23) as well as the carbonyl oxygen of the C-25 acetoxy group in rifampicin and amino acid residues R409, S411, Q393, H406, D396 and F394 in RNA polymerase (Figure 3).

The lower activity of rifamycins against Gram-negative bacteria is most likely caused by weaker penetration of the antibiotics through the cell membrane. Additionally, the β subunit of the DNA-dependent RNA polymerase is susceptible to mutational alterations which increase the resistance of *M. tuberculosis* and other pathogens to rifamycins. Therefore, rifampicin is usually used along with other antibiotics. The transport and the resistance problems can be partially solved by structural modifications. However, because of the complex structure of the rifamycins the modifications have been limited mainly to the C-3 or C-4 positions of the aromatic unit.

1.1.3 The Biosynthesis of Rifamycin

The biosynthesis of rifamycins has been extensively studied by classical feeding experiments with isotopically labeled precursors, mutagenesis approaches and complementary genetic and biochemical experiments.[8,24–26] All the reported studies demonstrated that rifamycins are synthesized by type I polyketide synthase (PKS) assembled from AHBA (**7**) as a starting building block through chain extension by eight propionate and two acetate units (Scheme 3).

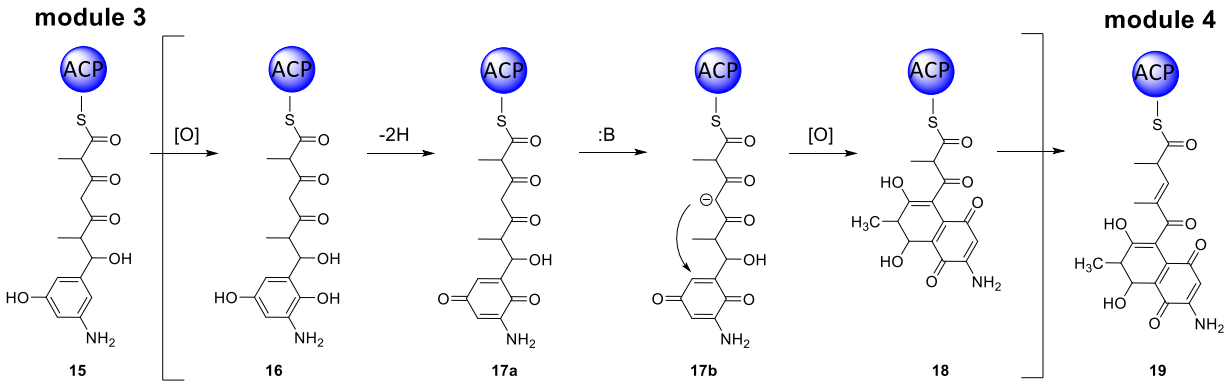


Scheme 3. Proposed biosynthesis of rifamycin B (**1**) in *A. mediterranei*[2] (AD = adenylation/thiolation didomain; KS = ketosynthase; AT = acyltransferase; DH = dehydratase; KR = ketoreductase; ACP = acyl carrier protein; *not active domain).

3-Amino-5-hydroxybenzoic acid (**7**) is recognized by a loading module which was identified as a didomain homologous to adenylation/thiolation domain (AD) of a non-ribosomal peptide synthetase (NRPS). The type I *rif*-PKS comprises ten modules arranged in five

multifunctional proteins which are encoded by the *rif*A-*rif*E genes. The product of the *rif*F gene - an amide synthase, releases a linear undecaketide from the PKS and catalyzes an intramolecular amide formation to give the macrocyclic lactam **14**.[2] Proansamycin X (**14**) is then processed to rifamycin B in a few steps which is described in the second part of this section.

Studies on the disruption of the *rif*F gene resulted in the isolation of a series of different chain length polyketides ranging from the tetra- to decaketides.[27–29] The deactivation of the *rif*D gene in module 8 and the *rif*E in modules 9 and 10 also revealed the formation of such linear ketides. These experiments provided clear evidence that the *rif*-PKS operates in a processive manner. Additionally, the structures of the isolated compounds suggested that the oxidative cyclization from a benzenoid to a naphthalenic structure must occur between the third and the fourth chain extension steps (Scheme 4).

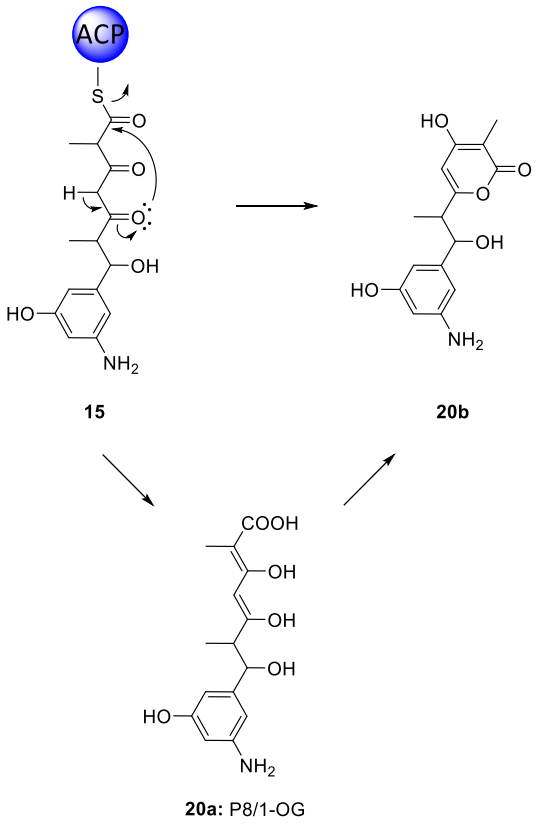


Scheme 4. Proposed mechanism of the oxidative cyclization from a benzenoid to a naphthalenic structure between the third and the fourth chain extension steps.

Whereas the tetraketide **15** bears an unmodified benzenoid structure, the penta- to decaketides have undergone a ring closure to the naphthoquinones. This transformation requires an enzymatic oxidation of the compound **15** to the hydroquinone **16** which is further oxidized to the quinone **17a**. The intramolecular Michael addition initiated by the deprotonation of the 1,3-diketo moiety, followed by reoxidation yields the dihydronaphthoquinone **18** which is processed to pentaketide **19** in the module 4.

Floss and co-workers investigated a number of genes which could be involved in this sequence of steps and indicated *rif-orf*19 as a gene implicated in the formation of the naphthalene ring.[30] It encodes a protein homologous to 3-(3-hydroxyphenyl)propionate hydoxylases – flavoproteins which catalyze the hydroxylation of phenol molecules. Similar

genes have been reported in the biosynthesis of many natural products, for example geldanamycin (**4**). The deactivation of *rif-orf*19 in a mutant strain resulted in a disruption of rifamycin B production and release of either the tetraketide P8/1-OG (**20a**) or its lactone form **20b** (Scheme 5), first isolated by Ghisalba in 1981.[8] Floss proposed that Rif-Orf19 acts as a separate protein interacting with the *rif*-PKS by introduction of a hydroxyl group into the ACP-bound tetraketide.

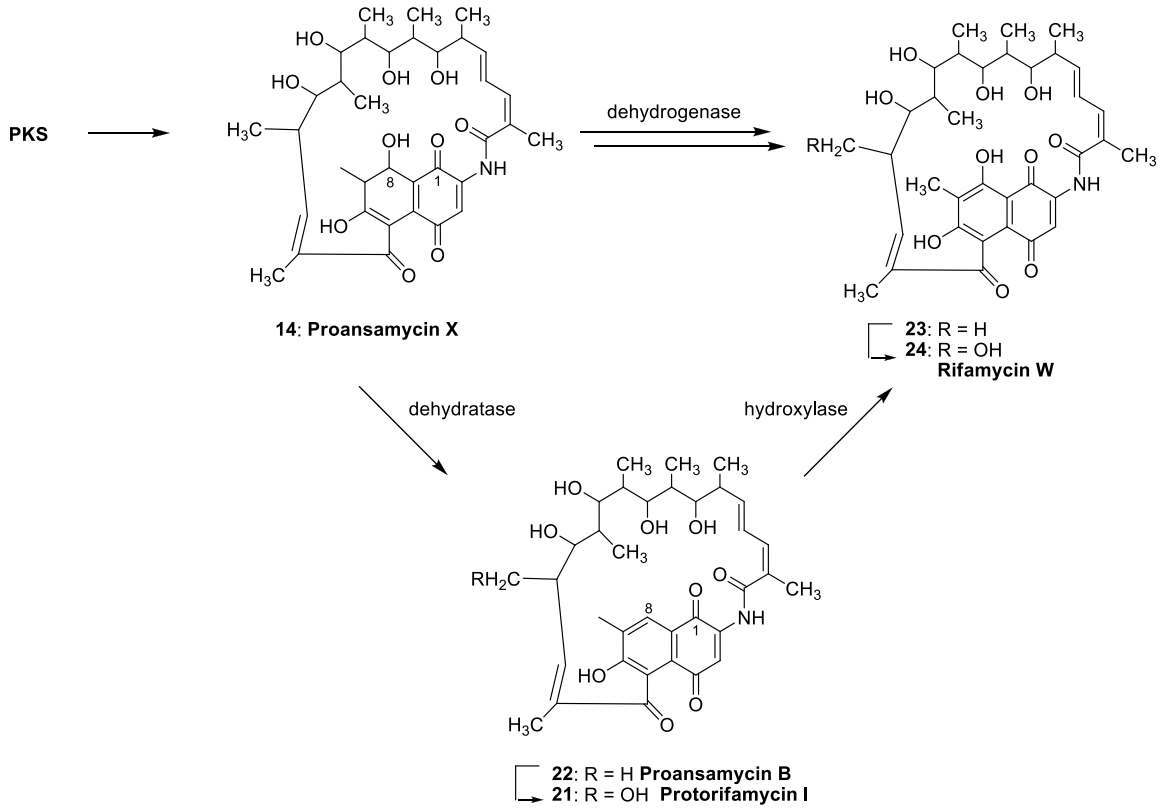


Scheme 5. Formation and release of the tetraketide **20** after premature polyketide chain termination in the mutant strain.

The naphthalene ring formation is unprecedented in polyketide biosynthesis. In the literature no examples of an oxidation domain located in the PKS system or oxidation reactions taking place during the polyketide assembly have been reported so far.

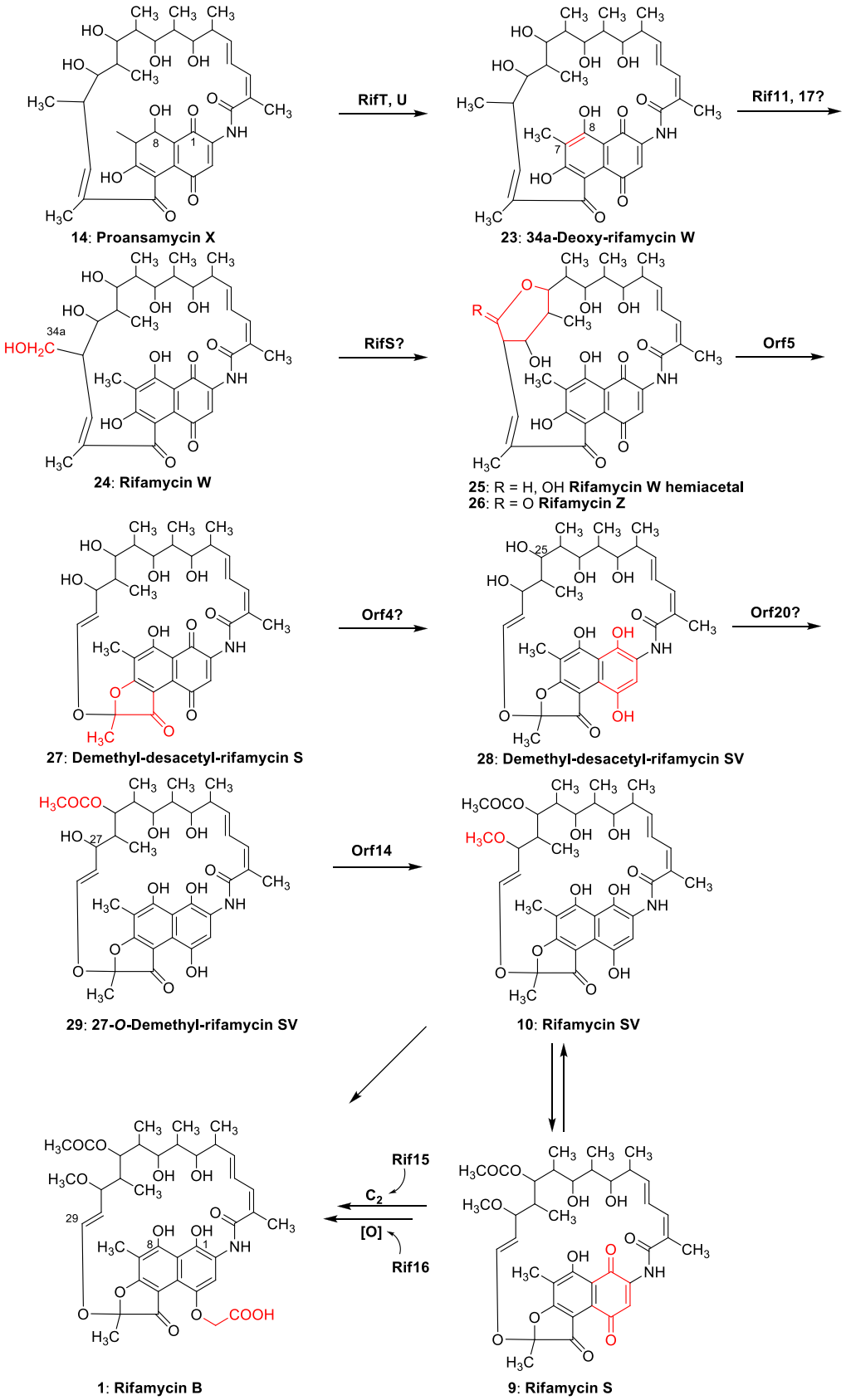
Proansamycin X (**14**) is the first macrocyclic product released from the *rif*-PKS. However, the structure of the earliest cyclic intermediate in rifamycin B biosynthesis has been investigated over the years. Based on the results of feeding experiments Ghisalba and Nüesch proposed protorifamycin I (**21**) as the first released product - a naphthoquinone lacking the 8-hydroxyl group.[31] This theory was refuted by Rickards in 1989 and reexamined by Floss in 1999.[27,32]

Rickards and co-workers carried out feeding experiments using [carboxy-^{13}C, ^{14}C, $^{18}O_2$]-AHBA and proved that the C-8 carbon atom and C-8 phenolic hydroxyl group originate from the carboxyl group of AHBA. Additionally, fermentation of *A. mediterranei* was carried out under an artificial atmosphere containing N_2 and $^{18}O_2$ providing clear evidence that the bacteria utilize atmospheric oxygen to introduce the C-1 oxygen function and the C-29 vinyl ether group. A few years later Floss and co-workers investigated whether the 8-hydroxynaphthoquinone structure in the rifamycin core is formed from the 8-hydroxy-7,8-dihydronaphthoquinone unit by dehydration followed by hydroxylation at C-8 or by a direct dehydrogenation to give the 7,8-double bond (Scheme 6). Feeding with protorifamycin I (**21**) to a mutant strain carrying all the downstream-processing genes resulted in no formation of rifamycin B (**1**). Therefore, the results obtained by Floss and Rickard support a pathway involving the dehydrogenation of proansamycin X (**14**) in the rifamycin biosynthesis.



Scheme 6. Possible routes from proansamycin X (**14**) to rifamycin W (**24**), the precursor of rifamycin B (**1**).

In 2002 Traber and co-workers isolated and characterized proansamycin B (**22**) which was converted to protorifamycin I (**21**), but the following transformation to rifamycin B (**1**) was not observed.[33]



Scheme 7. The role of individual *rif* genes in the rifamycin biosynthesis.[2]

However, the compound **21** was processed to 8-deoxyrifamycin B which suggests the presence of a minor shunt pathway in *A. mediterranei* alongside of the "normal" biosynthesis of rifamycin B.

The proposed biosynthetic pathway from proansamycin X (**14**) to rifamycin B (**1**) and the role of individual *rif* genes are depicted in Scheme 7. In the first steps proansamycin X (**14**) is converted to rifamycin W (**24**) by dehydrogenation to form the 7,8-double bond and the following oxidation at C-34a. Transformation of rifamycin W (**24**) to rifamycin SV (**10**)/rifamycin S (**9**) involves a number of oxidation steps, as well as acetylation and methylation. However, only limited information about the role of individual genes has been collected so far.

The role of the suggested *rif* biosynthetic genes has been deduced on the basis of sequence similarity analyses with other bacteria species which revealed a series of putative post-PKS modifying genes. It was found that the *rif* cluster contains genes homologous to P450-dependent monooxygenases, several regulatory genes and a group of genes encoding the formation of sugar nucleotides.[2] In 2003 Floss and co-workers isolated and characterized 27-*O*-demethylrifamycin SV methyltransferase, an enzyme encoded by the gene *rif orf14* and able to catalyze the *O*-methylation at C-27 of 27-*O*-demethylrifamycin SV (DMRSV, **29**) to give rifamycin SV (**10**).[34] The studies on the substrate specificity revealed that 27-*O*-demethylrifamycin SV methyltransferase converts only DMRSV, but not its quinone **27** nor its 25-*O*-deacetyl derivative DMDARSV (**28**). Additionally, the deactivation of the *rif orf14* gene supressed the ability to produce rifamycin B and resulted in accumulation of DMRSV. The gene encoding acetyltransferase which promotes a C-25-selective acetylation of the compound **28** to **29** could not be identified in the set of *rif* genes which suggests that it is not related to the *rif* biosynthetic gene cluster.

In 2011 the Zhao group published the results of their genetic studies on the *rif*15 and *rif*16 genes which were found to encode a transketolase and a P450 monooxygenase respectively, both of which are essential for the conversion of rifamycin SV (**10**) to rifamycin B (**1**).[35,36] The expression of these two genes in a mutant of *A. mediterranei* U32 producing rifamycin SV in the wild form[37] resulted in the conversion of the compound **10** to the desired product **1**. However, heterologous expression of *rif*15 and *rif*16 in *Streptomyces coelicolor* and *Mycobacterium smegmatis* failed to convert rifamycin SV to rifamycin B. These results may suggest that some other genes specific for *A. mediterranei* are involved in this transformation.

It is interesting to note that in 2007 Fenical and co-workers isolated a new group of bicyclic polyketides from the marine organism *Salinispora arenicola* called saliniketals which display striking structural similarities and identical stereochemistry to the ansa chain of the rifamycins (Figure 4).[38] They are used in the cancer chemoprevention as inhibitors of ornithine decarboxylase induction. Saliniketals A and B (**30**-**31**) and rifamycins (**1**, **10**, **28**, **29**) co-occurred in the fermentation mixture which together with genetic studies suggested the horizontal transfer of the *rif* genes between *A. mediterranei* S699 and *S. arenicola*. The biosynthetic similarity between these two groups of compounds was investigated by carrying out bioinformatic studies, mutagenesis experiments, studies with isotope-labeled precursors and chemical analyses. As a result, the saliniketals were found as the unexpected byproducts of the *rif* pathway diverging at the stage of 34a-deoxy-rifamycin W (**23**). The primary amide is formed in a retro-Michael cleavage of the C-N bond in one of the further steps.

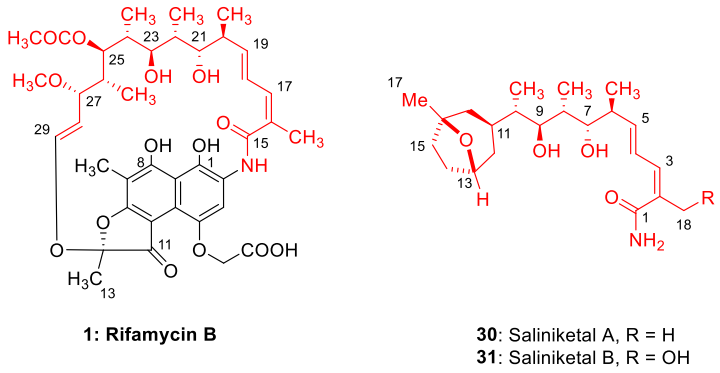


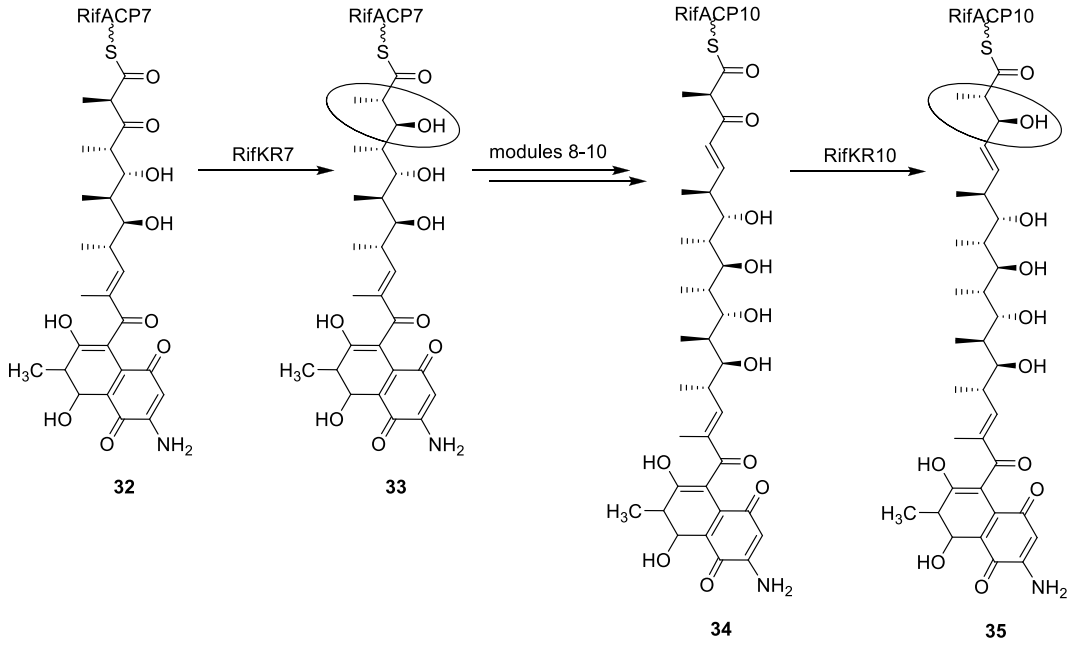
Figure 4. Structures of the rifamycin B (**1**) and the saliniketals isolated from *S. arenicola* CNS-205.

1.1.4 Stereospecificity of the Ketoreductase and Dehydratase Domains of Rifamycin Polyketide Synthase

Ketoreductases (KR) are one of the most versatile type I PKS enzymes which catalyze the reduction of the β-keto group in 2-methyl-3-ketoacyl acyl carrier protein (ACP) substrates to form an alcohol and in some cases also epimerization of the 2-methyl group.[39]

There have been only very few ketoreductases characterized in the literature so far.[40–43] In 2013 Cane and co-workers published the results of their studies on two KRs from the *rif*-PKS: RifKR7 from the module 7 and RifKR10 from the module 10. Incubation of (2*R*)-2-methyl-3-ketopentanoyl-EryACP6 as a model substrate with RifKR7 and NADPH resulted in the predominant formation of (2*S*,3*S*)-2-methyl-3-hydroxycyl-ACP product. A similar experiment with RifKR10 provided a product with the same stereochemistry as in the example mentioned

above. These results show that both enzymes catalyze the stereospecific epimerization of the 2-methyl group providing the (2*S*,3*S*)-2-methyl-3-hydroxycyl-ACP products **33** and **35**, respectively (Scheme 8).[44]



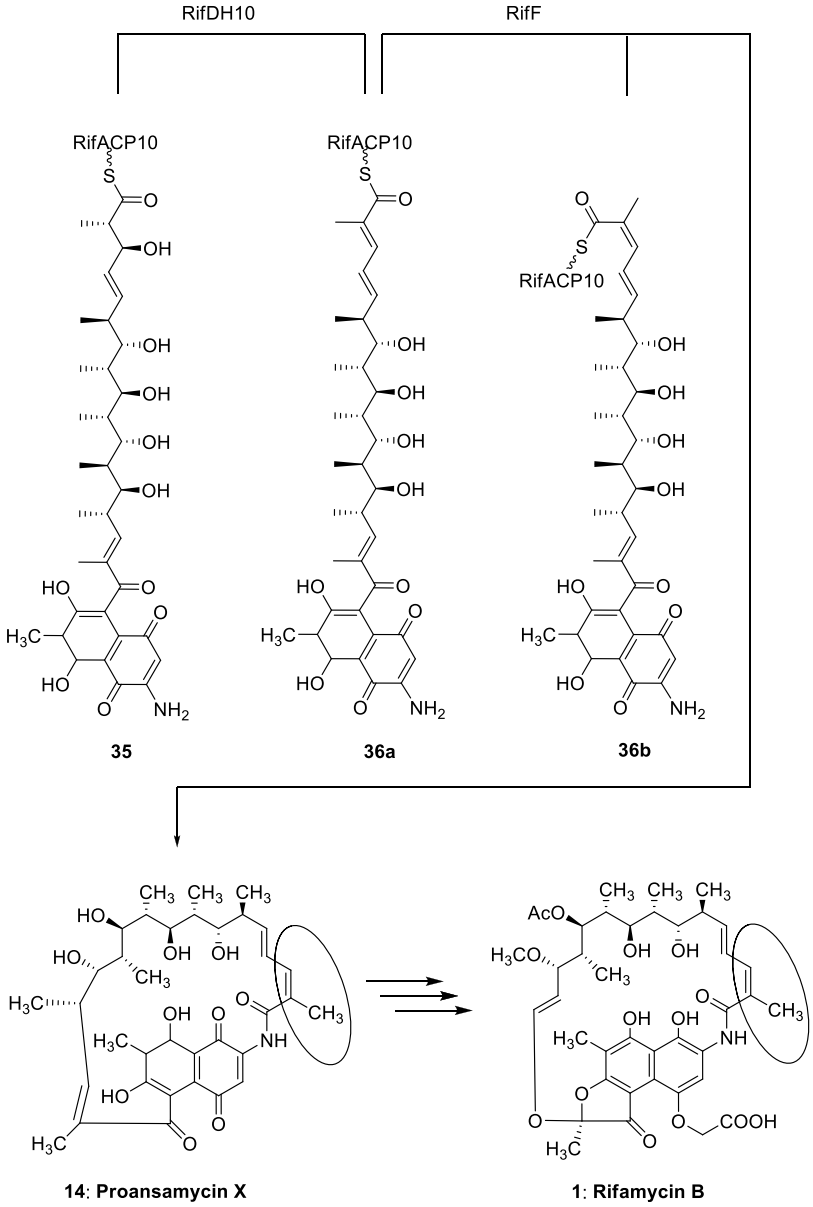
Scheme 8. Reactions catalyzed by RifKR7 and RifKR10 in the rifamycin biosynthesis.

Complementary studies have been carried out on the RifDH10 domain which uses the RifKR10-generated product as the substrate. Dehydratases (DH) are the PKS domains which catalyze elimination of the β-hydroxyl group and the α-proton to form an α,β-enoyl double bond. So far only two PKS DH domains have been experimentally determined by both obtaining the protein structure and investigation of the stereospecificity of the dehydration reaction. These are DHs from the erythromycin PKS (EryDH4)[45] and RifDH10 from the *rif*-PKS.[46]

RifDH10 is the dehydratase located in the terminal module 10 of the *rif*-PKS which was reported to catalyze the diastereospecific dehydration of RifACP10-bound (2*S*,3*S*)-2-methyl-3-hydroxyacyl undecaketide **35** resulting in the exclusive formation of (*E*)-2-methyl- 2-enoyl undecaketide **36a** (Scheme 9). Isomerization of the double bond to the *cis* configuration, characteristic of proansamycin X (**14**) and the final biosynthetic product rifamycin B (**1**), probably occurs during the macrolactamization step catalyzed by the amide synthase RifF.

To prove this hypothesis Cane and co-workers used four diastereomeric RifACP10-bound diketide thioesters as model substrates in recombination experiments. They demonstrated that

RifDH10 catalyzes dehydration of only one of them – (2*S*,3*S*)-2-methyl-3-hydroxypentenoyl-RifACP10 to give exclusively (*E*)-2-methyl-2-pentenoyl thioester product. This result highlights the stereospecific dehydration and substrate diastereoselectivity of RifDH10 in the acceptance and further processing of the ACP-bound undecaketide.



Scheme 9. Dehydration of a RifACP10-bound (2*S*,3*S*)-2-methyl-3-hydroxyacyl undecaketide **35** by RifDH10 and following macrolactamization of the linear product to proansamycin X (**14**), the rifamycin B (**1**) precursor.

Surprisingly, the substrate specificity of RifDH10 is reversed when the substrate is attached to a non-native acyl carrier protein, e.g. EryACP6 or the corresponding *N*-acetylcysteamine or

pantetheine thioester analogues resulting in dehydration of (2*R*,3*R*)-2-methyl-3-hydroxypentenoyl thioesters.

In spite of many structural similarities to EryDH4 and other characterized DH domains, the RifDH10 is unique in the sense of displaying strict specificity towards (2*S*,2*S*)-2-methyl-3-hydroxypentenoyl-RifACP10 substrate and catalyzing the dehydration of it. The opposite substrate specificity when RifDH10 is tethered to non-cognate ACP is also unusual and constitutes the first example published in the literature.

As it was shown in this section the rifamycins can be distinguished from other polyketides by a few unique features, including a naphthalene ring formation, a macrolactam ring and a trisubstituted *cis* double bond. Among over 2000 known polyketides carrying one or more double bonds, most of which have *trans* geometry, only a minor part contains one or more *cis* configured double bonds. Furthermore, in most PKSs the linear ketide is released from PKS by thioesterase (TE) which is replaced in the *rif*-PKS by the separately encoded amide synthase, RifF.

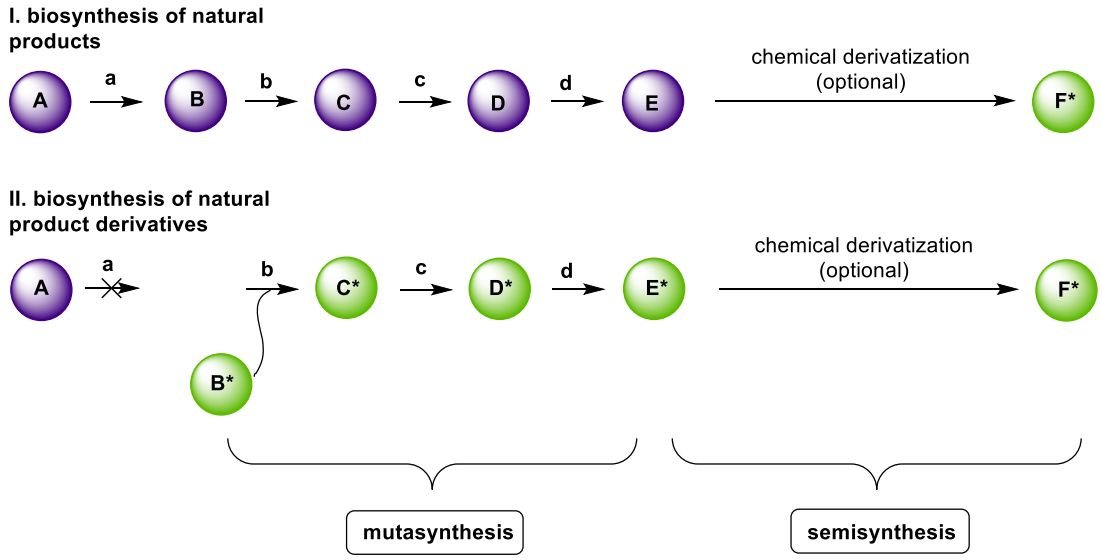
1.1.5 Mutasynthesis

Mutational biosynthesis or, in short, mutasynthesis is an attractive technique, alternative to semi- or total synthesis approaches, for the preparation of complex natural products with enhanced or unique biological activity. It combines the power of chemical synthesis with metabolic engineering and can be applied for creation of secondary metabolites libraries.[47–50] Mutasynthesis often complements semisynthesis and with respect to total synthesis it can be regarded as a biosynthetic shortcut.

The concept of mutasynthesis was first theoretically thought by Birch in 1963[51] and named by Rinehart in 1977 who defined it as a generation of a mutant strain of a producer organism which is blocked in the formation of a biosynthetic building unit of the target natural product. Feeding with chemically modified analogues of these building blocks, called mutasynthons, results in the production of new metabolites which are evaluated in terms of their biological activities (Scheme 10).[52]

Ansamycin antibiotics are perfectly suited for mutasynthetic studies as it has been shown by a few groups over the last ten years.[53–66] The libraries of new ansamycin antibiotics modified in the aromatic unit have been prepared by feeding AHBA analogues to the AHBA(−)

blocked mutants of *Streptomyces hygroscopicus*, the geldanamycin (**4**) producer[53,54], and *Actinosynnema pretiosum* which produces the ansamitocins.[55–64]

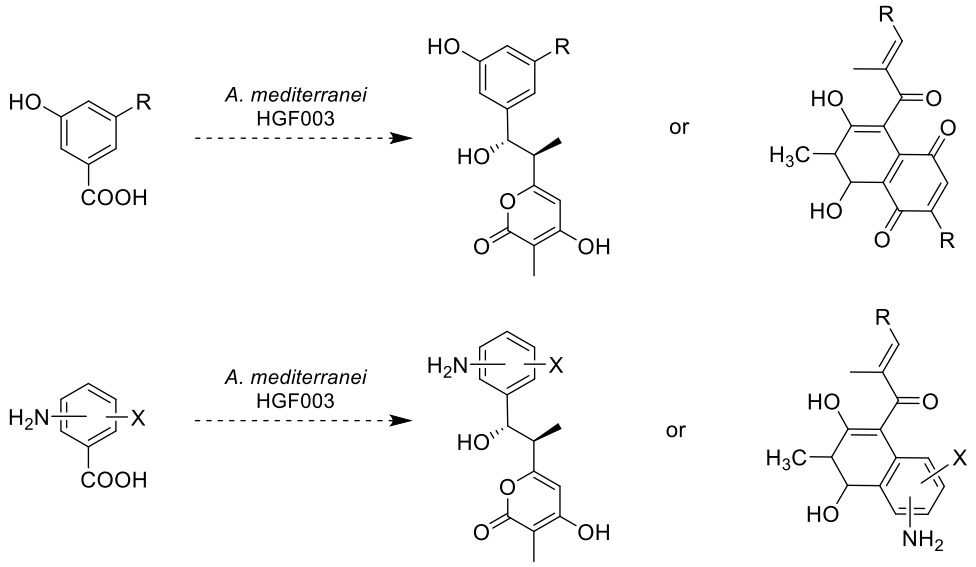


Scheme 10. A general concept of mutasynthesis and semisynthesis (a-d = enzymes, A = starting material, B-D = biosynthetic intermediates, E = natural product, B* = mutasynthon, C*-D* - new intermediates, E*, F* = generated derivatives of natural product).[47]

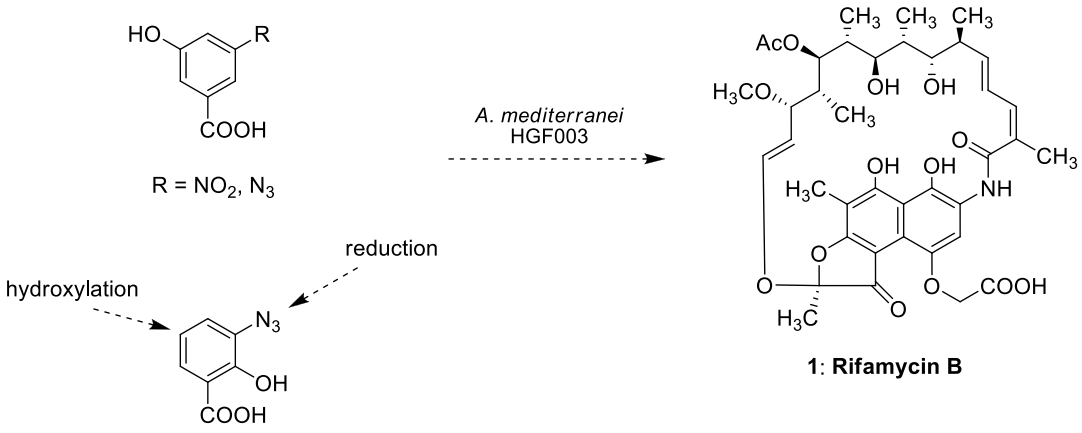
In spite of its advantages, mutasynthetic approach has a few clear limitations. First of all, feeding with modified building blocks to a mutant strain requires a broad substrate flexibility of all catalytic domains including the first enzyme loading the substrate onto the PKS. Some of the produced secondary metabolites lack structural complexity and in such cases the semi- or total synthesis approach can be considered. On the other hand, employing more complex substrates may hamper the acceptance by the producer organism and/or the transport through the cell membrane. Additionally, the technique cannot be applied for the mutants blocked in the biosynthesis of natural precursors when the starter unit is essential for the growth of the producer organism. Some of these problems may be solved by means of metabolic engineering, namely the relocation of enzymes to heterologous organisms. The substrate specificity may be widened by changing the respective modules to their analogues from other gene clusters which display a broader spectrum of selectivity. The yield and growth of the producer strain can be improved by optimization of the fermentation procedures.

1.2 Purpose of the Project: Mutasynthetic Approach Towards New Rifamycin Derivatives

The aim of the first project is to obtain and identify new rifamycin derivatives using an AHBA(−) blocked mutant of *Amycolatopsis mediterranei* HGF003 and AHBA analogues as the substrates. These mutasynthetic studies are a continuation of the previous work on AHBA mutants of *Streptomyces hygroscopicus*, a geldanamycin producer and *Actinosynnema pretiosum*, an ansamitocin P3 producer.



Scheme 11. Planned feeding experiments with AHBA analogues and AHBA(−) blocked mutant of *A. mediterranei* HGF003.



Scheme 12. Reductive properties of *A. mediterranei* planned to be tested with nitro and azido analogues of AHBA.

Aromatic building blocks are planned to be supplemented to the mutant strain HGF003 in order to evaluate its synthetic potential, the scope of tolerance and limitations. The mutasynthons are divided into two groups: substituted *m*-hydroxybenzoic acids and substituted aminobenzoic acids bearing electron-withdrawing groups (Scheme 11).

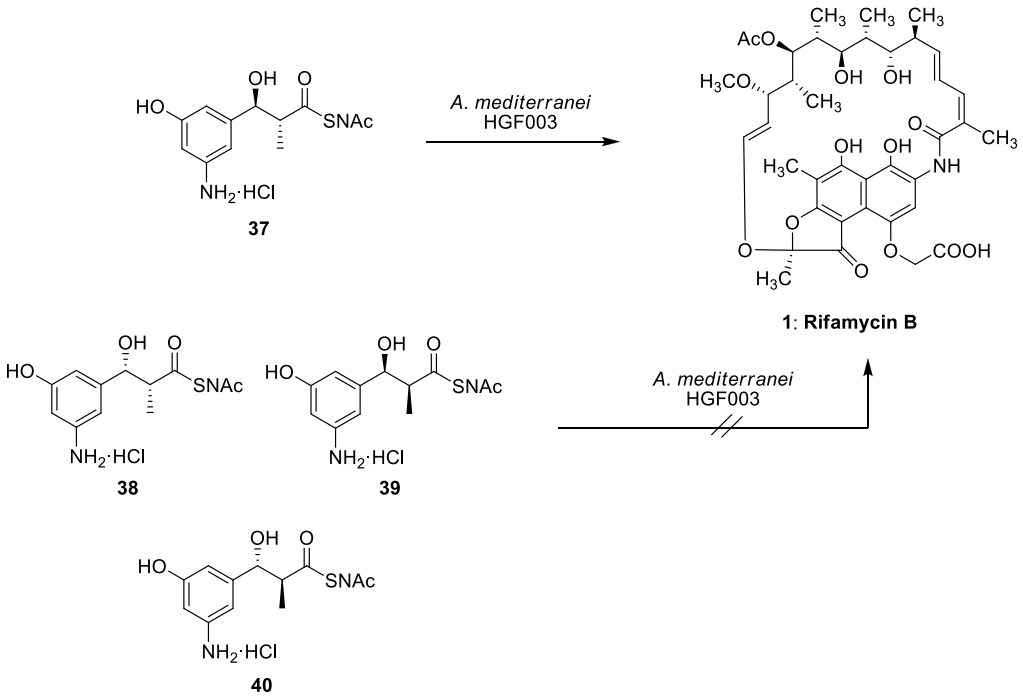
Additionally, the reductive properties of *A. mediterranei* HGF003 will be investigated. In order to do that, a few nitro and azido analogues of AHBA will be fed to the mutant strain (Scheme 12).

1.3 Discussion and Results

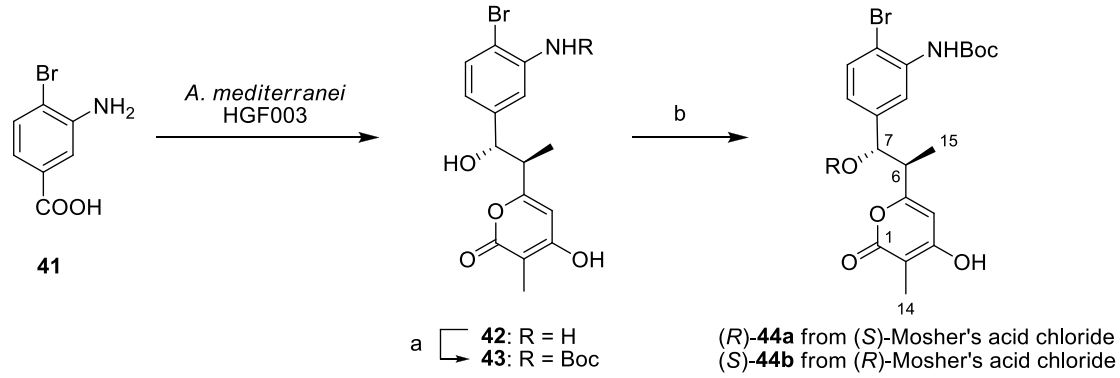
1.3.1 Previous Work on Structure Elucidation of the Stereochemistry at C-6 and C-7 in Tetraketides

Stereogenic centers generated in module 1 are also present in tetraketide **20b** but disappear during processing in the *rif*-PKS. Determination of the absolute configuration of both stereogenic centers at C-6 and C-7 in tetraketides has been reported by Khosla and co-workers.[67] They prepared four diastereomers of a biosynthetic substrate (**37**-**40**) for module 2 of the ri*f*-PKS in form of their SNAc esters which were fed to the blocked mutant of *A. mediterranei* (Scheme 13). HPLC and LC-MS analyses with rifamycin B (**1**) as a reference revealed that only the substrate **37** was transformed into the compound **1**.

In the Kirschning group bromotetraketide **42** has been chosen for the determination of the absolute configuration of both stereogenic centers at C-6 and C-7.[68,69] The pyranone **42** was obtained in the yield of 19 mg/L after feeding with 3-amino-4-bromobenzoic acid **41** to the mutant strain and subsequently transformed into Mosher esters **44a** and **44b** (Scheme 14). The stereogenic center at C-7 was determined to be *S* on the basis of ^{1}H NMR analyses, while the configuration at C-6 was established to be *R* by carrying out computational analyses of conformational energies using SPARTAN ’08 (Wavefunction, Inc., Irvine, CA, USA). The latter analysis was based on the relationship between the H-H coupling (J = 8.7 Hz) of the vicinal protons at C6 and C-7, as observed in the ^{1}H NMR spectrum, and the torsion angle (179.4°). Both stereochemical assignments - at C-6 and C-7 are in agreement with the results reported by Khosla.[67]



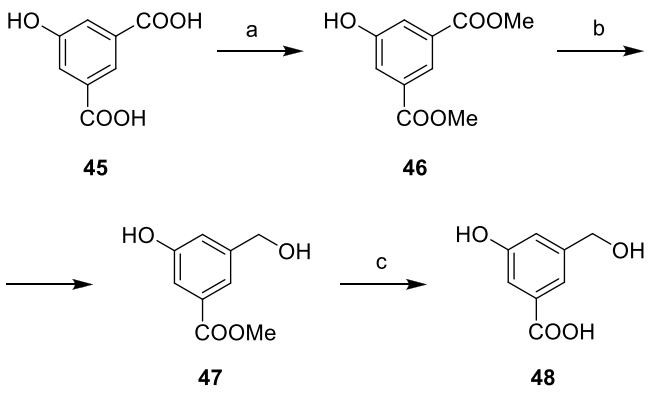
Scheme 13. *In vivo* transformations with four diketide analogues.



Scheme 14. Stereochemical assignment of C6 and C7 after Mosher esterification.[68] Reaction conditions: a) Boc_2O, Et_3N, dioxane/water, RT, 12 h, 49%; b) (*S*)-(+)-MTPA-Cl or (*R*)-(−)-MTPA-Cl, Et_3N, DMAP, CH_2Cl_2, RT, 24 h, 50%.

1.3.2 Synthesis of Mutasynthons

The synthesis of 3-hydroxy-5-(hydroxymethyl)benzoic acid (**48**) was first reported by Simone Eichner[70] and modified using 5-hydroxyisophthalic acid (**45**) as a starting material (Scheme 15). The compound **45** was transformed into a methyl ester **46** under standard esterification conditions employing methanol and concentrated sulfuric acid. The reaction was carried out under refluxing conditions for 20 h and provided the compound **46** in 97% yield.

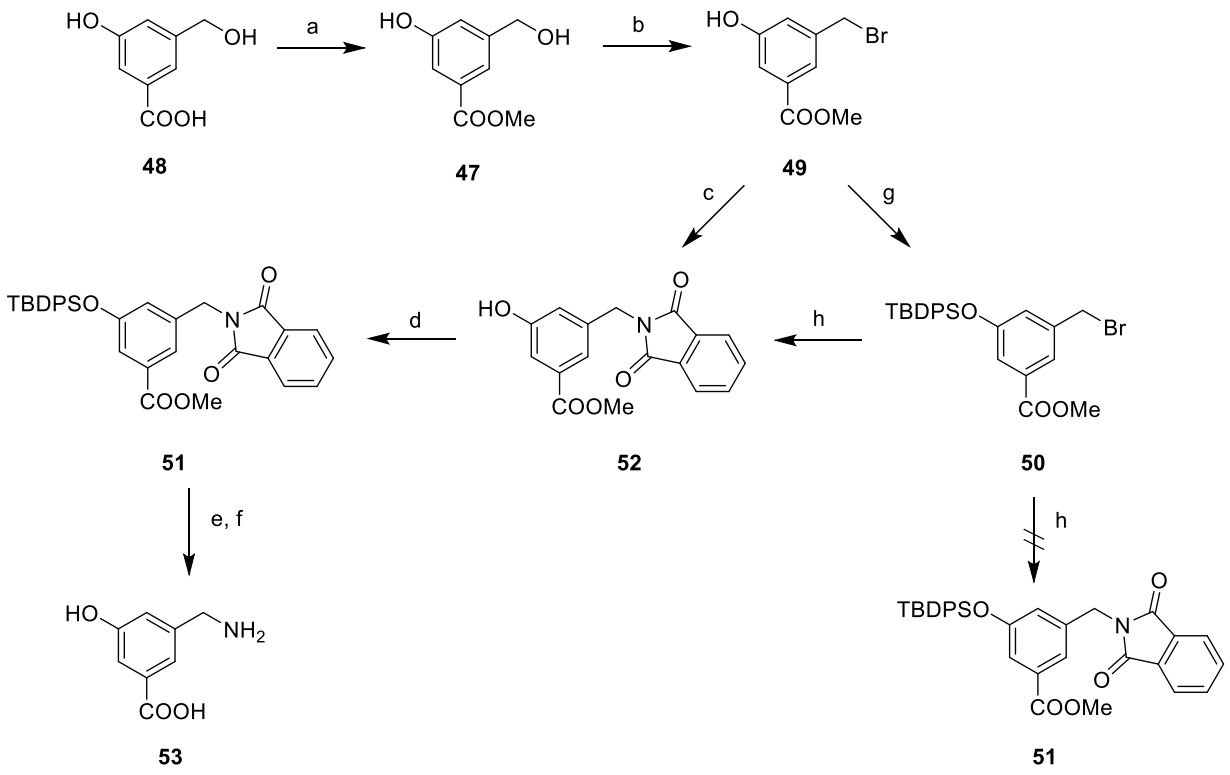


Scheme 15. Synthesis of 3-hydroxy-5-(hydroxymethyl)benzoic acid (**48**). Reaction conditions: a) MeOH, conc. H_2SO_4, reflux, 20 h, 97%; b) $LiAlH_4$, THF, 0 °C → RT, 3.5 h, 44%; c) LiOH (1 M in H_2O), MeOH, RT, 2 h, 96%.

One of the ester groups of dimethyl 5-hydroxyisophthalate (**46**) was then reduced using 1 M solution of $LiAlH_4$ in THF according to the Portnoy's procedure[71] to give the corresponding benzyl alcohol **47** in 44% yield. The last step was saponification carried out in MeOH with the use of a 1 M aqueous solution of LiOH providing the desired benzoic acid **48** in 96% yield.

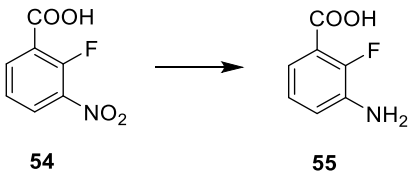
The next mutasynthon, 3-(aminomethyl)-5-hydroxybenzoic acid (**53**) was prepared using the above described methyl 3-hydroxy-5-(hydroxymethyl) benzoate (**47**) or 3-hydroxy-5-(hydroxymethyl)benzoic acid (**48**) as starting materials (Scheme 16).

Esterification of **48** with methanol in the presence of sulfuric acid provided the compound **47** in quantitative yield. The subsequent Appel reaction yielded methyl 3-(bromomethyl)-5-hydroxybenzoate (**49**) which was protected in the next step with TBDPSCl providing the compound **50** in 97% yield. The following step - substitution with phthalimide potassium salt was expected to give the compound **51** but unexpectedly yielded the TBDPS-deprotected product **52** which was directly obtained from benzyl bromide **49** in one of the previous experiments. The compound **52** had to be protected with TBDPSCl again due to troublesome work-ups in the next steps and submitted to the second part of the Gabriel synthesis involving hydrolysis with hydrazine monohydrate and work-up by acidic hydrolysis to give the desired benzoic acid **53** in quantitative yield.



Scheme 16. Synthesis of 3-(aminomethyl)-5-hydroxybenzoic acid (**53**). Reaction conditions: a) MeOH, conc. H_2SO_4, reflux, 24 h, quant.; b) CBr_4, PPh_3, CH_2Cl_2, RT, 3 h, 90%; c) Phthalimide potassium salt, K_2CO_3, DMF, RT, 3 h, 60%; d) Imidazole, DMAP, TBDPSCl, CH_2Cl_2, RT, 5 h, quant.; e) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 3 h; f) conc. HCl, reflux, 2 h; quant.; g) Imidazole, DMAP, TBDPSCl, CH_2Cl_2, RT, 5 h, 97%; h) Phthalimide potassium salt, K_2CO_3, DMF, RT, 3 h, quant. to **52**.

The synthesis of 3-amino-2-fluorobenzoic acid (**55**) from 2-fluoro-3-nitrobenzoic acid (**54**) has been already published in the literature by Baell and co-workers.[72] However, after loading of the substrate into the ThalesNano H-Cube® with a 10% Pd/C CatCart® they were not able to separate the obtained product **55** from a small residual amount of the substrate.

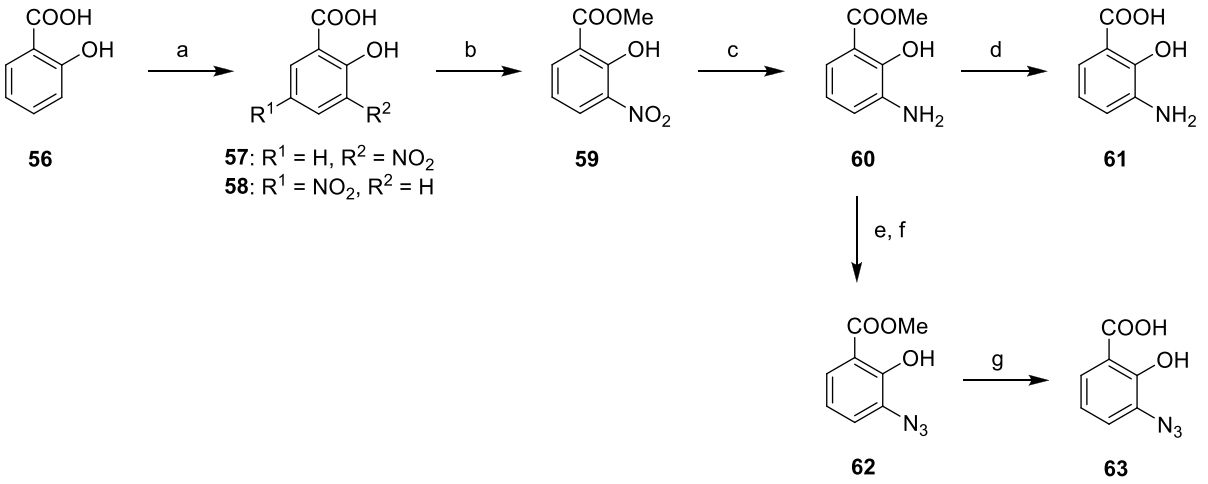


Scheme 17. Synthesis of 3-amino-2-fluorobenzoic acid (**55**). Reaction conditions: 10% Pd/C, TES, EtOH, RT, 1 h, 97%.

In this approach 2-fluoro-3-nitrobenzoic acid (**54**) was reduced to the desired 3-amino-2-fluorobenzoic acid (**55**) using molecular hydrogen generated *in situ* by addition of

triethylsilane to 10% Pd/C (Scheme 17). This simple approach has been developed by McMurray in 2007 and successfully employed in the reduction of azides, imines, nitro groups and multiple bonds, as well as in deprotection of benzyl and allyl groups.[73]

The syntheses of 3-amino-2-hydroxybenzoic acid (**61**) and 3-azido-2-hydroxybenzoic acid (**63**) were conducted using methyl 2-hydroxy-3-nitrobenzoate (**59**) which was prepared according to the procedure reported by Zeng (Scheme 18).[74]



Scheme 18. Syntheses of 3-amino-2-hydroxybenzoic acid (**61**) and 3-azido-2-hydroxybenzoic acid (**63**). Reaction conditions: a) HNO_3 (65%), conc. H_2SO_4, CH_2Cl_2, 0 °C, 30 min; b) MeOH, conc. H_2SO_4, reflux, 48 h, 47%.; c) 10% Pd/C, TES, EtOH, RT, 1 h, 94%; d) LiOH (1 M in H_2O), MeOH, 40 °C, 2 h, 88%; e) HCl (2 M), $NaNO_2/H_2O$, 0 °C; f) NaN_3, NaOAc, 0 °C, 2 h, quant.; g) LiOH (1 M in H_2O), MeOH, 40 °C, 2 h, 90%.

Nitration of salicylic acid (**56**) led to a mixture of benzoic acids **57** and **58** which were used in the following esterification step without further purification. The nitro compound **59** was obtained after purification by flash chromatography in 47% yield and subsequently reduced to the amine **60** in 94% yield using triethylsilane and 10% Pd/C. The following saponification with 1 M aqueous solution of LiOH provided 3-amino-2-hydroxybenzoic acid (**61**) in 88% yield. The compound **60** was converted *in situ* to a diazonium salt, following by treatment with sodium azide to yield the compound **62** quantitatively. The subsequent saponification of **62** provided the desired aryl azide **63** in 90% yield.

The remaining substrates used for the feeding experiments were either commercially available or prepared according to the known literature procedures (details described in the section 3.4).

1.3.3 Mutasynthetic Approach with a Blocked Mutant of *Amycolatopsis mediterranei* HGF003

The feeding experiments were carried out using AHBA(−) mutant of *A. mediterranei* HGF003 generated by a knockout of RifK activity (Figure 5) responsible for the aromatization of the AHBA precursor, 5-deoxy-5-amino-3-dehydroshikimic acid (Scheme 1). When AHBA (or some of its analogues as described in the further part of this thesis) is added to the production medium, the ability to biosynthesize the rifamycins is restored.

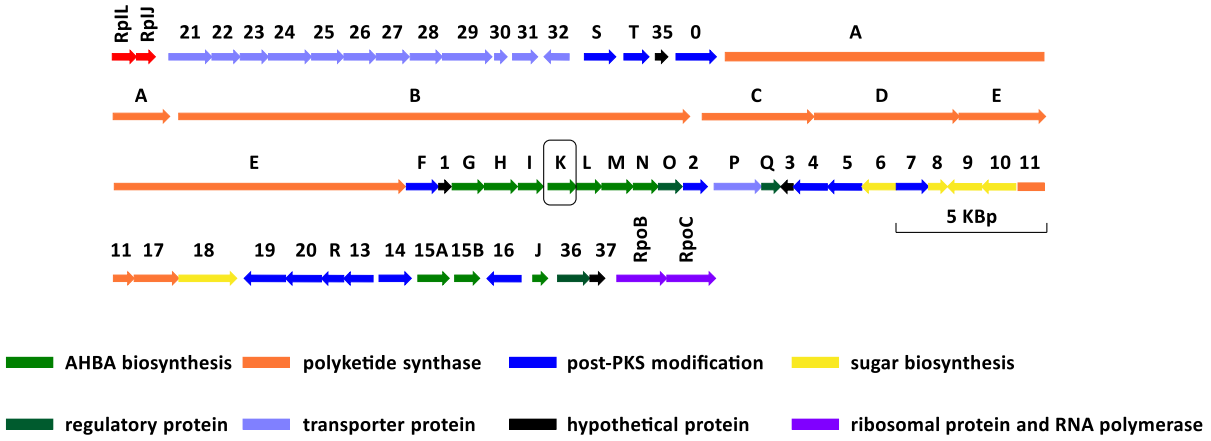


Figure 5. Biosynthetic gene cluster of *rif*-PKS and flanking genes encoding proteins and DNA-dependent RNA polymerase.

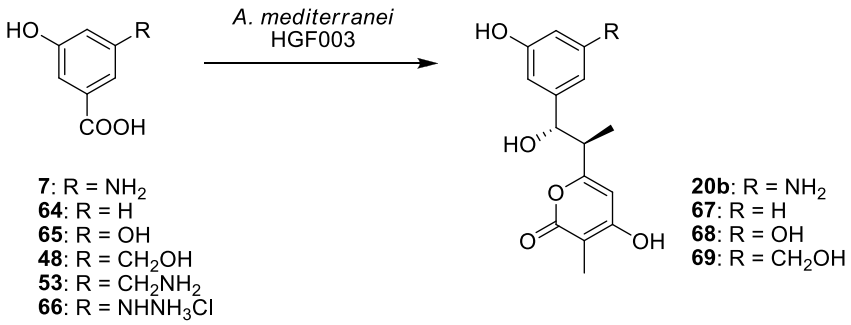
To analyze the flexibility of the loading domain in the *rif*-PKS, a series of benzoic acid derivatives has been supplemented to the mutant strain. Intramolecular cyclization of the β-ketothioester leading to dihydronaphthalenes, similar to the one presented in Scheme 4 (Section 1.1.3), was sought to be achieved by appropriate choice of either phenols, which could be oxidized to hydroquinones (like **15** → **16**) or electron-withdrawing groups (NO_2, CN, CF_3, halides, ester group).

Every fermentation process was conducted with an additional flask containing AHBA (positive control) and with no substrate added (zero control) to monitor the productivity of the bacterial strain and exclude a false-positive result, respectively.

1.3.4 Mutasynthetic Experiments with Substituted *m*-Hydroxybenzoic Acids

The first feeding experiment with *A. mediterranei* HGF003 was conducted using the natural starter unit, AHBA (**7**) to optimize the fermentation conditions and to control the productivity of the strain. As expected, cultivation of the strain in the presence of AHBA resulted in

restoration of rifamycins biosynthesis. When fermentation was carried out in liquid medium, rifamycin B (**1**) and rifamycin S (**9**) were isolated in the amounts of 20 mg/L and 1.1 mg/L, respectively (Table 1). Additionally, traces of the released tetraketide **20b** were detected by UPLC-MS. Cultivation on solid agar medium for the same period of time resulted in the production of rifamycin S (2.4 mg/L) and rifamycin W (12.8 mg/L).[69] The extension of the fermentation period could presumably lead to the production of rifamycin B or rifamycin S but this hypothesis was not investigated.



Substrate	Rifamycin B **1** (mg/L)	Rifamycin S **9** (mg/L)	Rifamycin W **24** (mg/L)	Pyranone (mg/L)
7	20[a]	1.1[a] 2.4[b]	12.8[b]	**20b**[c]
64	–	–	–	**67** (118)
65	–	–	–	**68** (4.2)
48	–	–	–	**69** (chemically labile)
53	–	–	–	not formed
66	–	–	–	not formed

Table 1. Products isolated after supplementation of AHBA(−) blocked mutant of *A. mediterranei* with different *m*-hydroxybenzoic acids. [a] fermentation was carried out in liquid medium; [b] fermentation was carried out on solid agar medium; [c] Detected by UPLC-MS: *m/z*: $[M+H]^+$ calcd for $[C_{15}H_{18}NO_5]^+$: 292.1185, found: 292.1182.

Mutasynthetic approach with *A. mediterranei* HGF003 strain has been reported in the literature with the use of only two AHBA analogues: 3-hydroxybenzoic acid (**64**) and 3,5-dihydroxybenzoic acid (**65**).[75] These experiments were repeated resulting in the formation of the known 2*H*-pyran-2-ones **67** and **68**. The tetraketide **67** was isolated in preparatively excellent yield (118 mg/L) which makes it useful as a chiral building block in further synthesis of more complex molecules. Unfortunately, hydroxylation and the following

hydroquinone or naphthoquinone formation were not observed. It remains unknown whether the release of the tetraketide from the module 3 is a result of a spontaneous liberation due to the presence of the labile diketo carbonyl system or its enols forms or if there is an enzyme which presents a blockade to the polyketide chain elongation. It seems that oxidation and hence cyclization, as well as the presence of an aromatic amino group are the necessary conditions for further processing by the enzymes of the *rif*-PKS.[69]

The next mutasynthons employed in the feeding experiments were the benzyl alcohol **48** and the benzylamine **53**. This approach was based on the results observed in the geldanamycin producer, *S. hygroscopicus* which was able to process the alcohol **48** to ring-enlarged macrolactones.[76] In this case, the supplementation of AHBA(−) mutant of *A. mediterranei* with the compound **48** led to the formation of the chemically labile pyranone **69**. The corresponding benzylamine **53** was not processed and only the detoxification products could be detected (the amino group was *N*-acylated). Also was the aryl hydrazinium chloride **66** not tolerated by the bacteria and no products could be found in the crude fermentation mixture.[69]

1.3.5 Mutasynthetic Experiments with Substituted Aminobenzoic Acids

The studies on the flexibility of the loading domain in the *rif*-PKS have been extended to differently substituted and functionalized benzoic acids (Figure 6).[69] In the first group of the aminobenzoic acids fed to the mutant strain has neither anthranilic acid (**70**) nor its nitro-substituted derivatives **71-72** been accepted by bacteria. Except for the compound **70** these results are in agreement with the kinetic data collected for the loading module by Khosla and co-workers.[77] They expressed and purified adenylation-thiolation didomain and carried out kinetic measurements for the covalent loading of the A-T didomain by different unnatural substituted benzoic acids. The rate constants for the T domain arylation were measured relative to the T domain arylation by benzoic acid.

4-Aminobenzoic acids **73-74** were also not processed by the mutant strain of *A. mediterranei*. Subsequently, a series of differently functionalized 3-aminobenzoic acids has been prepared and used in the further feeding experiments. The 2-hydroxysubstituted mutasynthon **61** was not loaded onto the *rif*-PKS resulting in the formation of the detoxification products, while 3-amino-2-flurobenzoic acid (**55**) was processed to the pyranone **87** in unexpectedly good yield of 25 mg/L (Figure 7).

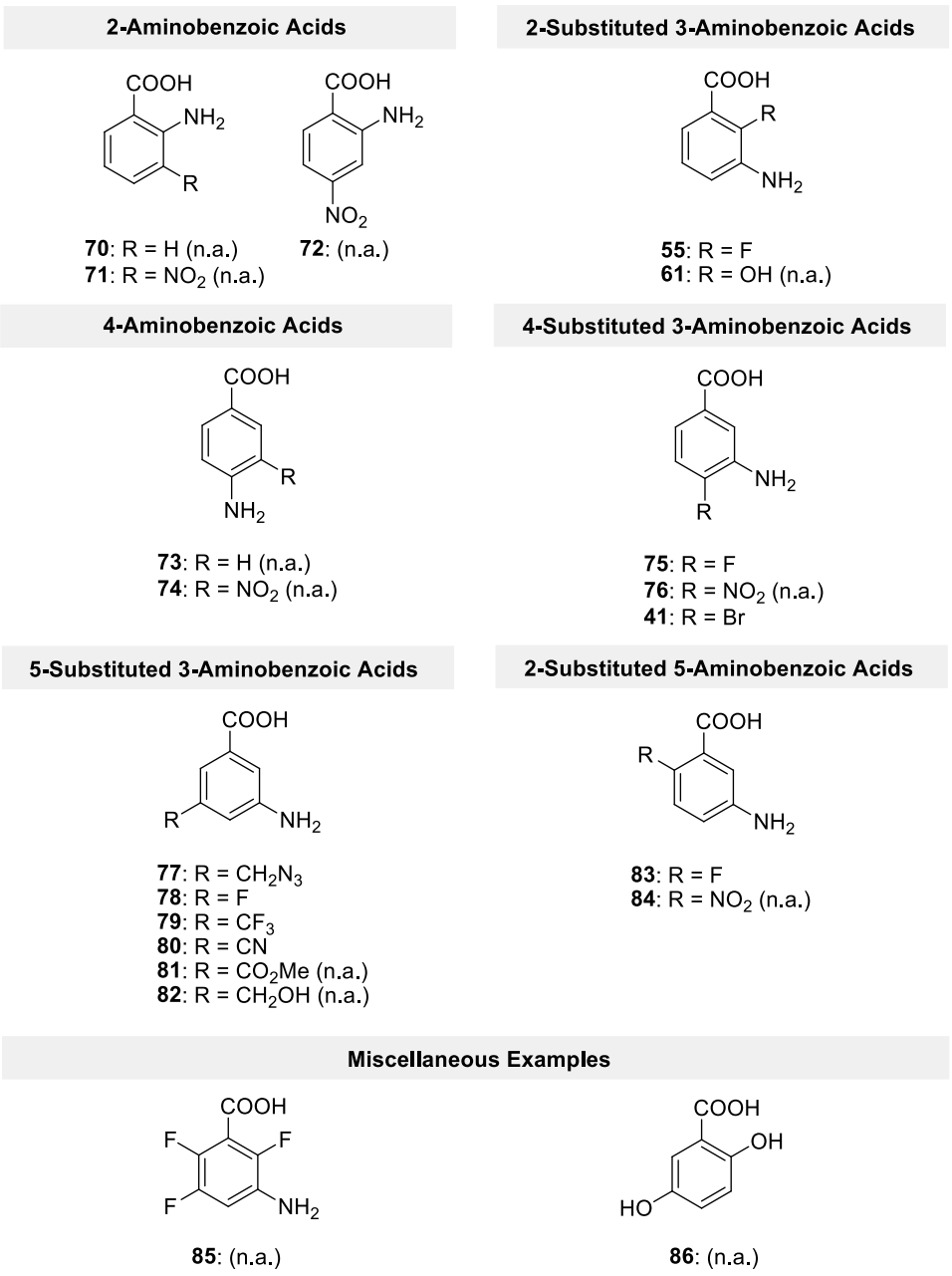


Figure 6. Aminobenzoic acids used for supplementation of AHBA(−) blocked mutant of *A. mediterranei*. X, Y = different substituents; n.a. = not accepted but detoxification products were detected (benzamides and acetylated amines listed in the section 4.2).

Similarly, 3-amino-4-nitrobenzoic acid (**76**) was not consumed by the mutant strain, whereas 4-fluoro- and 4-bromosubstituted compounds **75** and **40** were accepted and transformed into the tetraketides **88** and **42**, isolated in the satisfying amounts of 20 mg/L and 19 mg/L, respectively.

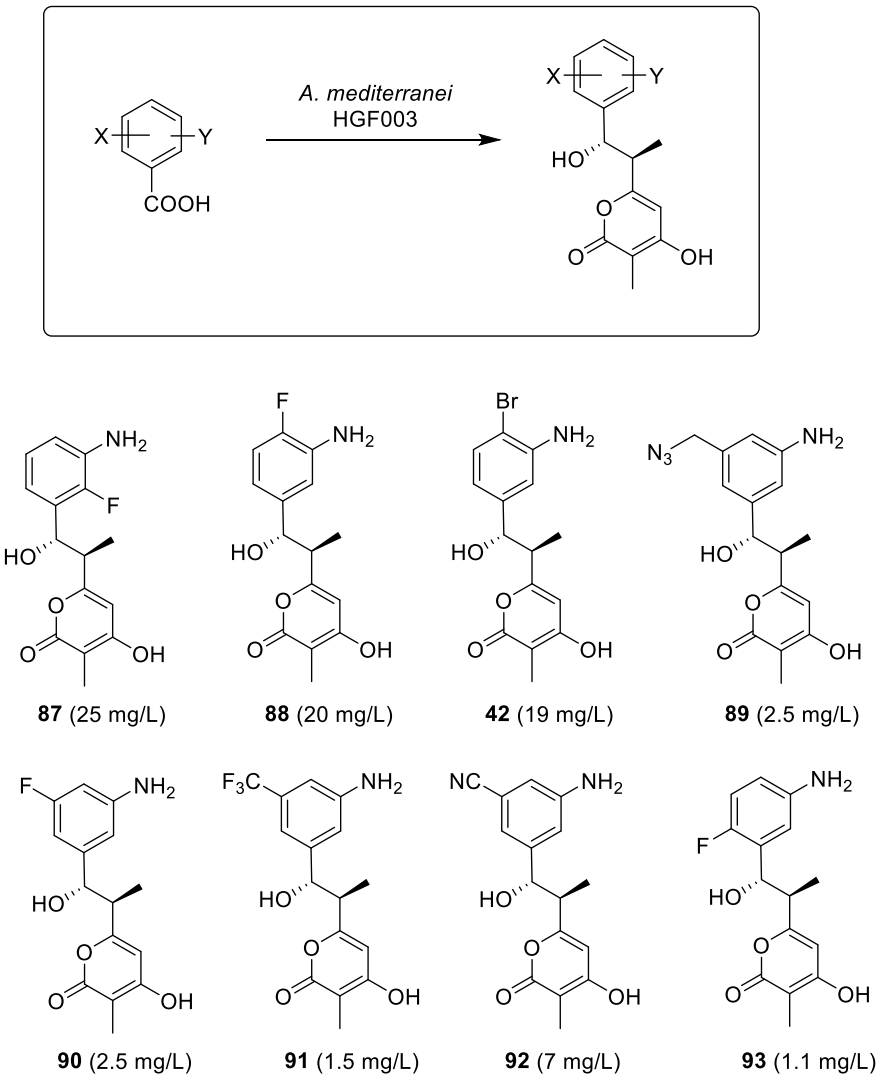


Figure 7. Products isolated after supplementation of AHBA(−) blocked mutant of *A. mediterranei* with aminobenzoic acids. X, Y = different substituents.

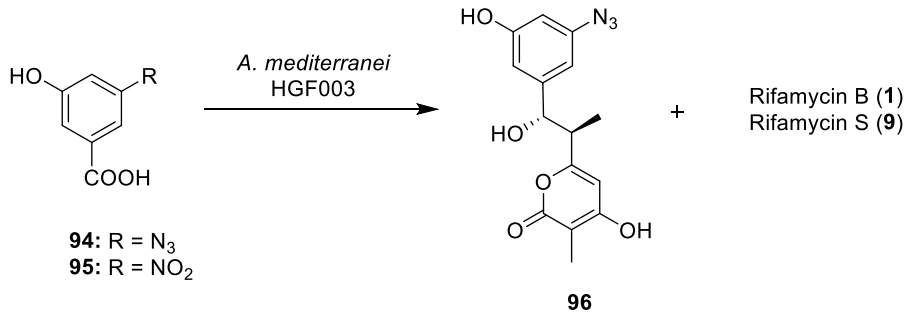
Interestingly, the 3-aminobenzoic acids with a third substituent located at position 5, as in mutasynthons **77**-**82**, are processed preferentially with good yields. These results are in accordance with the already mentioned kinetic data.[77] The 5-azidomethyl- and 5-fluropyranones **89**-**90** were isolated in the amount of 2.5 mg/L. Contrary to expectations, the presence of electron-deficient functionalities, that is a trifluoromethyl group in the mutasynthon **79** and a nitrile group in the compound **80**, did not promote cyclization to the corresponding dihydronaphthalenes. However, the substrates were processed to the stage of the tetraketides isolated subsequently in the yields of 1.5 mg/L and 7 mg/L, respectively, before their release from the *rif*-PKS. The last two mutasynthons from this group: the compound **81** with an ester group and the benzyl alcohol **82** were not accepted by the mutant strain.

Additionally, it is worth emphasising that all the fluorine-bearing mutasynthons **55**, **75** and **83**, which contain a substitution pattern (functionalizations in the positions 2-, 4- and 6-) not favored in the active site of the loading domain (AT_L-ACP_L) are transformed into the corresponding 2*H*-pyran-2-ones **87**, **88** and **93**, respectively. Presumably, the fluorine atom is sufficiently small atom to occupy the aromatic ring in the binding site of the AT_L-ACP_L, similarly to the unsubstituted 3-aminobenzoic acid[77] or 3-hydroxybenzoic acid (**64**) (Table 1). Additionally, fluorine-containing compounds are very promising for the medicinal chemistry as the fluorine atom has been reported to improve drug properties.[78]

1.3.6 Studies on the Reductive Properties of *Amycolatopsis mediterranei*

Mutasynthetic studies on the geldanamycin producer, *S. hygroscopicus*, revealed its unique and unprecedented reductive properties towards aryl azides, a few examples of which were transformed into the corresponding anilines.[57] The *A. mediterranei* HGF003 was found to be another bacterial strain able to reduce the azido group, not only to the amino group but also to the nitro group.[69]

After feeding of the AHBA(−) blocked mutant of *A. mediterranei* with 3-azido-5-hydroxybenzoic acid (**94**) a 2*H*-pyran-2-one derivative **96** was isolated as the main product in the amount of 47 mg/L (Table 2).



94: R = N_3
95: R = NO_2

96

Mutasynthon	Rifamycin B **1** (mg/L)	Rifamycin S **9** (mg/L)	Pyranone **96** (mg/L)
94	2.0	1.3	47.0
95	–	Detected by UPLC-MS[a]	–

Table 2. Products isolated after supplementation of *A. mediterranei* with 3-azido-5-hydroxybenzoic acid (**94**) and 3-hydroxy-5-nitrobenzoic acid (**95**). [a] UPLC-MS detection: *m/z*: $[M+H]^+$ calcd for $[C_{37}H_{46}NO_{12}]^+$: 696.3020, found: 696.3022.

Additionally, rifamycin B and rifamycin S were obtained in the yields of 2 mg/L and 1.3 mg/L, respectively. Both rifamycins could be formed only after prior reduction of the aryl azide to the aryl amine which was processed further by the *rif*-PKS. The formation of the tetraketide **96** indicates that this reduction takes place before the PKS processing which is in agreement with results obtained for *S. hygroscopicus*.[57] Alternatively, the reduction of the compound **96** may take place in competition with its release, while the reduced product is transformed into rifamycins.

Supplementation of the mutant strain with 3-hydroxy-5-nitrobenzoic acid (**95**) resulted in the formation of rifamycin S (**9**) in trace amounts as detected by UPLC-MS. It has been the first example of a nitroarene reduced to an aryl amine by the ansamycin producer. Additionally, no pyranone has been detected in the fermentation extract. The chromatograms of the crude fermentation extracts after supplementation with 3-azido-5-hydroxybenzoic acid (**94**) and 3-amino-5-hydroxybenzoic acid (**7**) as a positive control are presented in Figure 8. A similar result was obtained after feeding of 3-hydroxy-5-nitrobenzoic acid (**95**, Figure 9) to the growing culture. The peaks visible in each case at the same retention time (ca. 3.2 min) have been assigned to the mass of rifamycin B ($[M\text{-}H]^-$ = 754). The chromatogram was recorded also for a zero control (fermentation medium without any mutasynthon) in which there were no peaks at the retention time t_R = 3.2 min or for the mass of rifamycin B 756 in the positive mode and 754 in the negative mode.

The characterization of rifamycins via mass spectrometry has been well described in the literature.[79–81] However, HPLC with a tandem mass spectrometry LC-MS/MS system for the identification of rifamycins has been reported for the first time by Hewavitharana in 2007.[82] The fragmentation of a 755 *m/z* ion → 273 *m/z* ion was monitored during the characterization of rifamycin B (**1**) and rifamycin SV (**10**) in various strains of the marine sponge-derived bacterium *Salinispora*.

The presence of a 273 *m/z* ion (a so-called chromophoric ion) corresponds to a naphthofuran system (Figure 10) which is the most useful ion indicative of rifamycins and provides clear evidence for the presence of these compounds.[82] It is formed by the loss of an acetate group and the subsequent removal or fragmentation of the ansa group. The 273 *m/z* ion was also observed in the MS/MS spectrum of rifamycin B recorded at the collision energy of 30-45 eV (Figure 11) after feeding with mutasynthons **94** and **95**. In both cases the fragmentation pattern was the same.

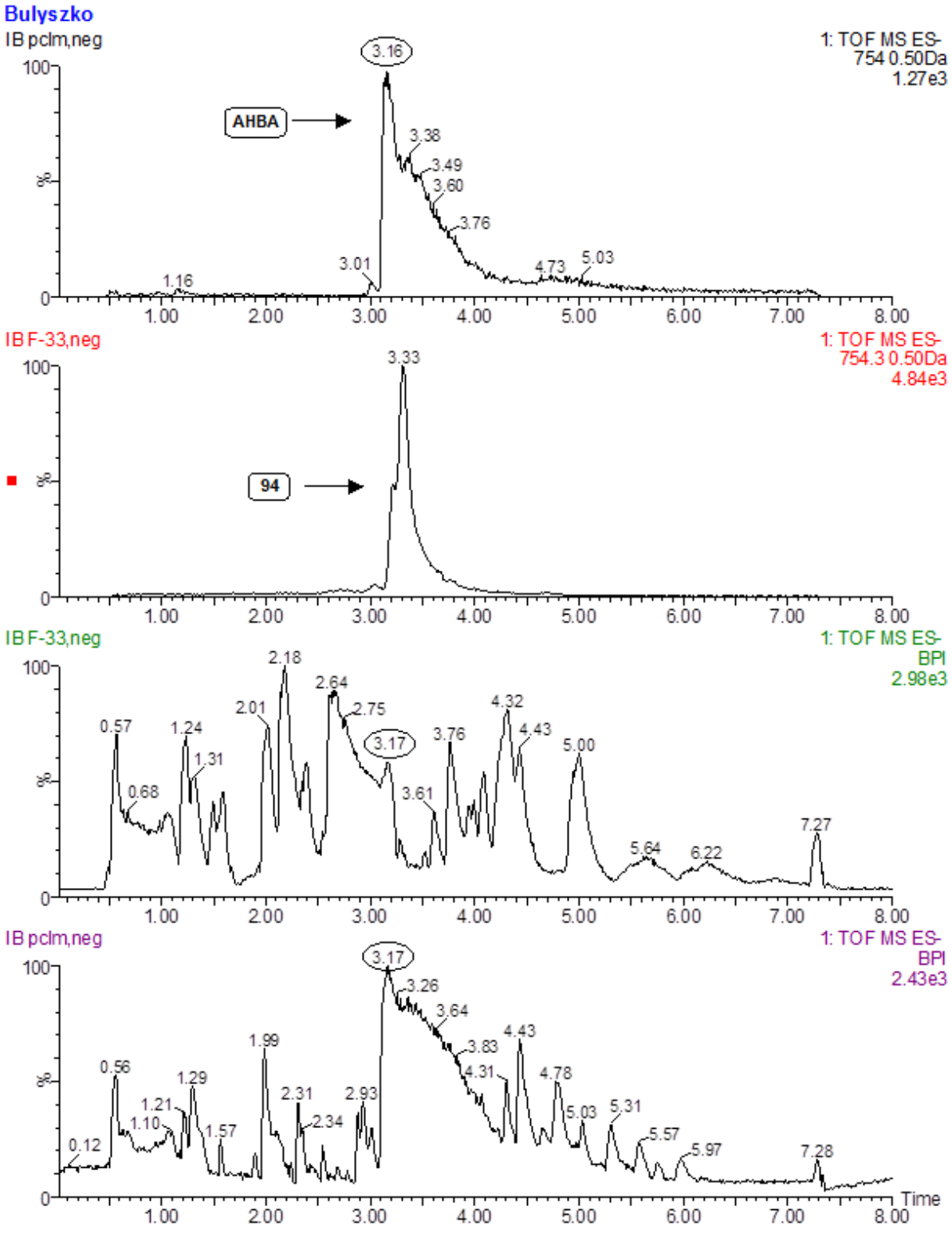


Figure 8. Chromatograms of the crude fermentation extracts after feeding with 3-azido-5-hydroxybenzoic acid (**94**, IB F-33) and AHBA (**7**) as a positive control (IB pclm): $[M\text{-}H]^-$ = 754; t_R = 3.2 min.

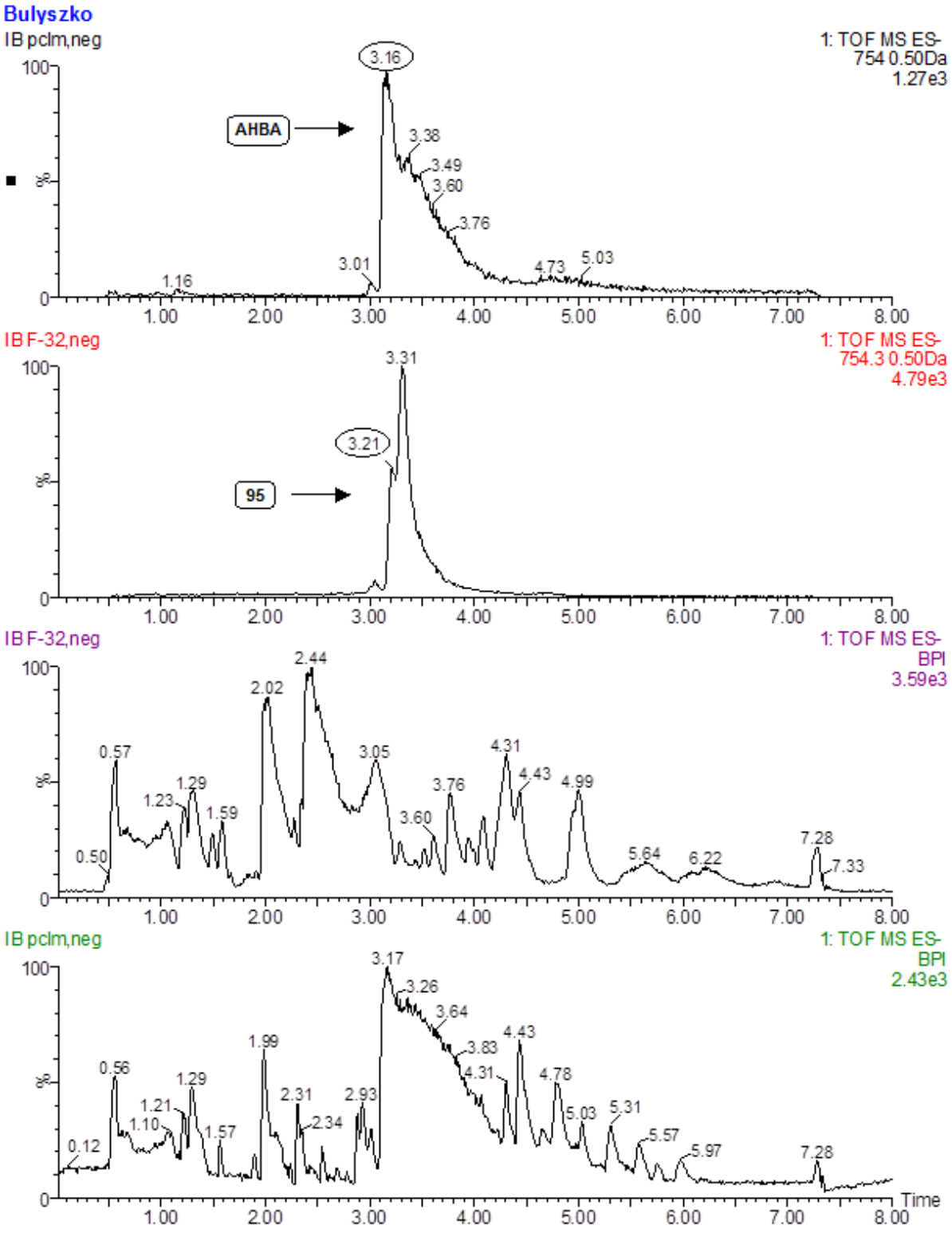


Figure 9. Chromatograms of the crude fermentation extracts after feeding with 3-hydroxy-5-nitrobenzoic acid (**95**, IB F-32) and AHBA (**7**) as a positive control (IB pclm): $[M\text{-}H]^-$ = 754; t_R = 3.2 min.

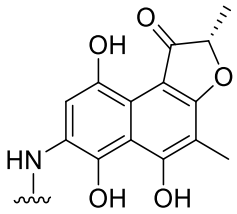


Figure 10. The naphthofuran system observed at 273 *m/z*.

Another abundant ion in the low mass region of the MS/MS spectrum was observed at 151 *m/z*. As suggested by Zerilli, it may originate from the ansa chain of rifamycins.[79] In the high mass region of the spectrum no significant peaks were observed – noteworthy are a low-intensity signal at 756 *m/z* corresponding to the $[M+H]^+$ of rifamycin B and a 724 *m/z* corresponding to the loss of methanol.

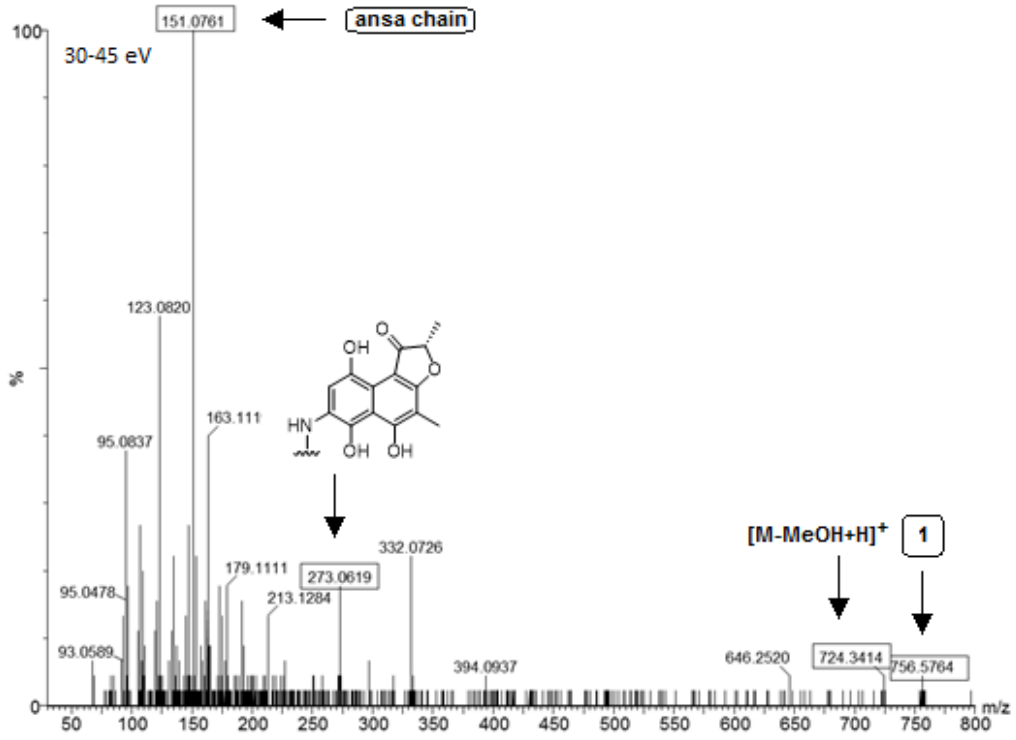


Figure 11. Fragmentation pattern of rifamycin B in MS/MS spectrum recorded at the collision energy of 30-45 eV.

An MS/MS spectrum of rifamycin B recorded at the collision energy of 15-30 eV showed a lower degree of fragmentation (Figure 12). In the high mass region of the spectrum a low-intensity signal of 756 *m/z* was observed while the most abundant ions 724 *m/z* and 664 *m/z* correspond to the loss of methanol and the following loss of acetic acid, respectively.

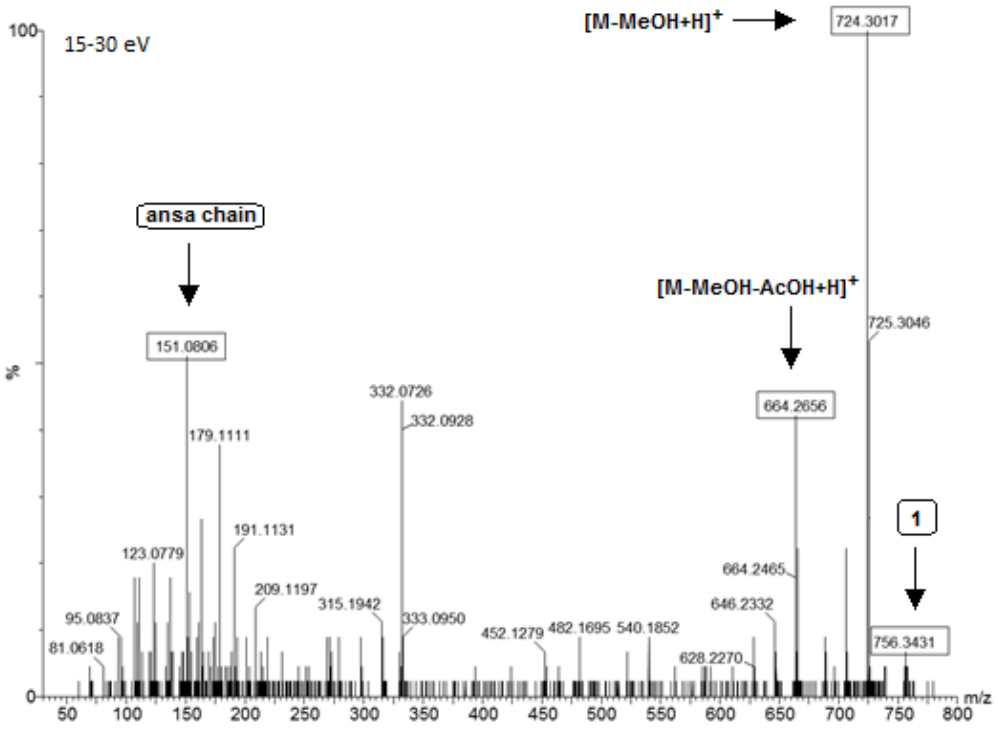


Figure 12. Fragmentation pattern of rifamycin B in MS/MS spectrum recorded at the collision energy of 15-30 eV.

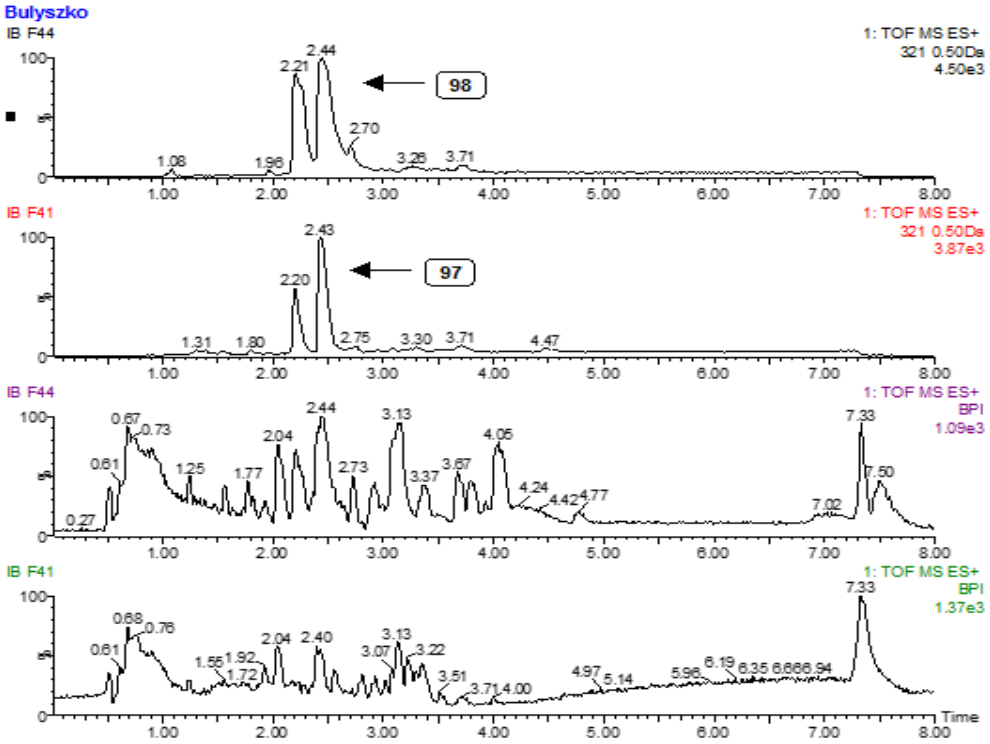
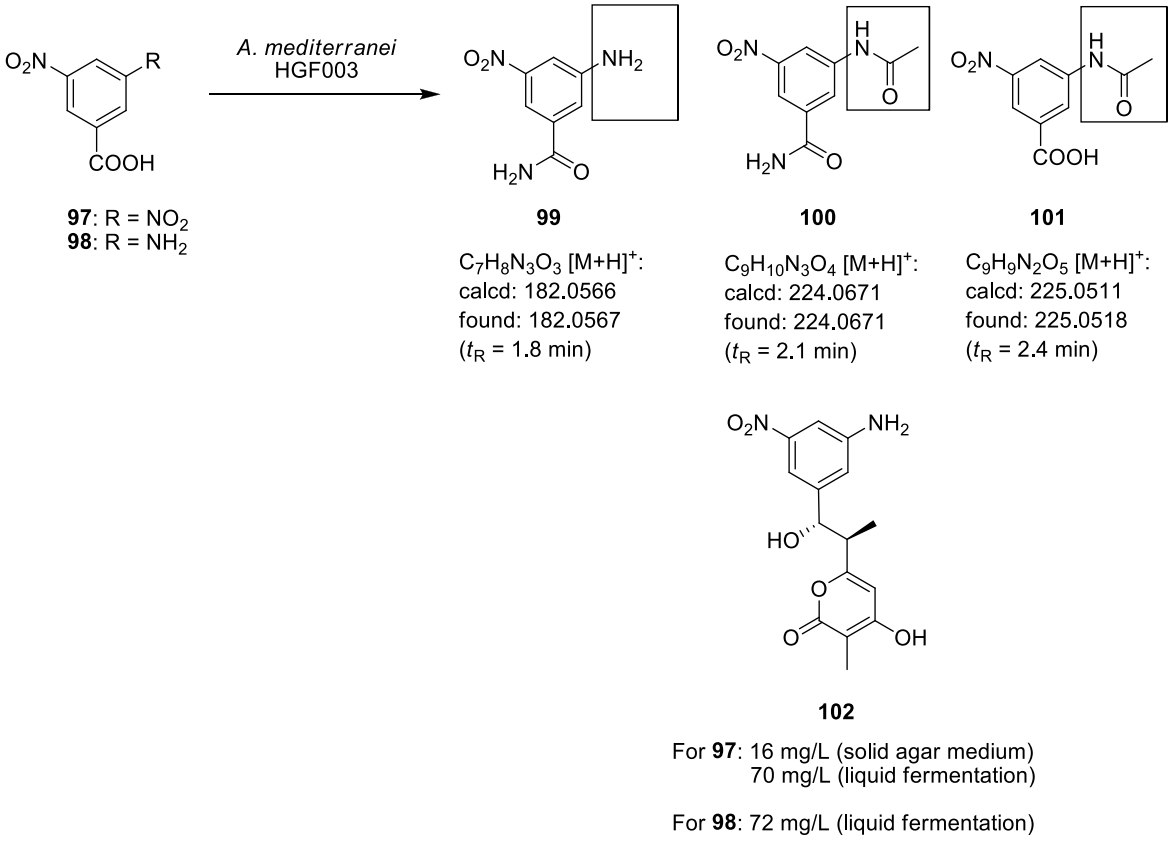


Figure 13. Chromatograms of the crude fermentation extracts after feeding with 3,5-dinitrobenzoic acid (**97**, IB F41) and 3-amino-5-nitrobenzoic acid (**98**, IB F44): $[M+H]^+$ = 321; t_R = 2.44 min.

It was observed during the HPLC purification steps that the amount of rifamycin S (**9**) in the fractions containing rifamycin B was gradually increasing which was most likely caused by exposure to light and air. These observations were also reported by Hewavitharana.[82] Rifamycin B can be synthetically converted to its other forms, e.g. to rifamycin SV which can be further oxidized to rifamycin S.

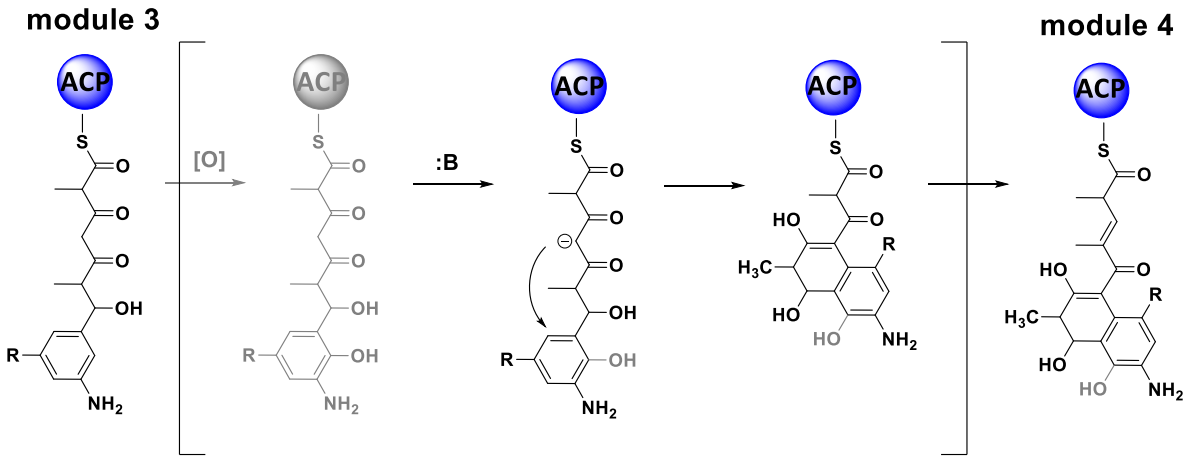


Scheme 19. Reductive properties of *A. mediterranei* after supplementation with nitrobenzoic acids **97** and **98**.

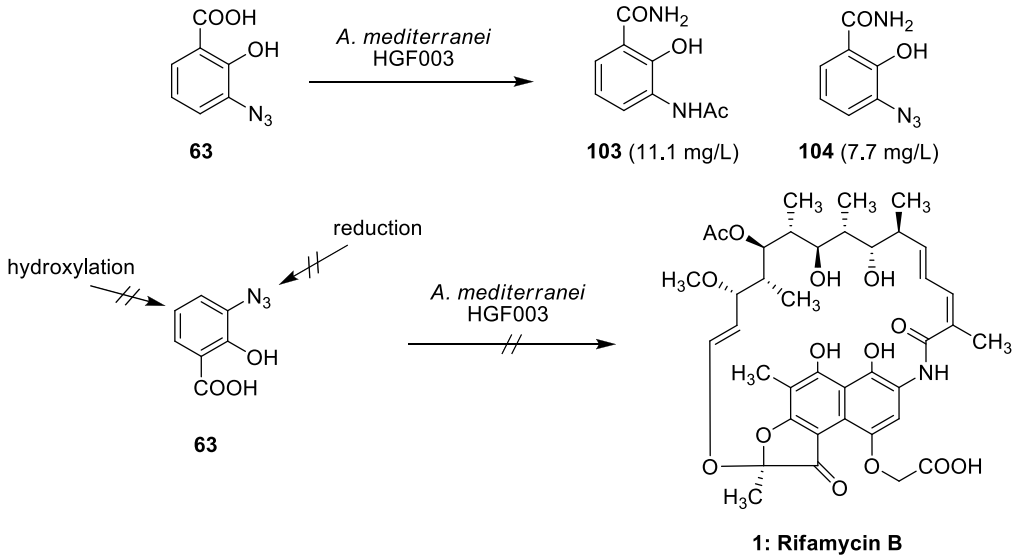
A. mediterranei displayed more impressive reductive properties towards nitro groups when it was fed with 3,5-dinitrobenzoic acid (**97**) resulting in the formation of the tetraketide **102** with one amino and one nitro group (Scheme 19). Interestingly, the pyranone **102** was isolated in a similar yield (ca. 70 mg/L) after supplementation of 3-amino-5-nitrobenzoic acid (**98**) to the mutant strain (Figure 13). Additionally, in both fermentation extracts two detoxification products **99** and **100** could be detected by UPLC-MS. The reduction of only one nitro group results in the formation of an aminonitroarene which is different from dinitro- or diaminoarenes and therefore possesses different binding properties for the cryptic enzyme and is no longer a suitable substrate for further processing by *rif*-PKS. Surprisingly, these results

do not go in parallel with the kinetic data which found 3,5-dinitrobenzoic acid (**97**) to be rejected by the loading domain due to steric reasons.[77] In the kinetic measurements for arylation of the loading domain carried out by Khosla and co-workers, also 3-nitrobenzoate and 3-sulfobenzoate were rejected as substrates for the A-T didomain.

The formation of the tetraketides **96** and **102** confirms that the presence of electron-withdrawing groups (a nitro group in this case or trifluoromethyl and nitrile groups in the mutasynthons **79**-**80**, respectively), is not sufficient to promote a vicarious nucleophilic substitution reaction and therefore to lead to the formation of a dihydronaphthalene unit (Scheme 20). When compared to the proposed mechanism of the oxidative cyclization from a benzenoid to a naphthalenic structure depicted in Scheme 4, one can notice that in case of mutasynthons bearing electron-withdrawing groups only first oxidation may take place. The further processing towards the desired pentaketide is blocked either at the oxidation step or at the cyclization step via an intramolecular vicarious nucleophilic substitution reaction. However, while none of the expected products were detected during LC-MS analyses, the hypothesis of a direct release of the tetraketide from the module 3 in a lactone form is supported.



Scheme 20. Proposed mechanism of the formation of a hypothetical pentaketide after feeding of the mutasynthons bearing electron-withdrawing groups (R = NO_2, CF_3, CN, CO_2Me, F) to the AHBA(−) mutant strain of *A. mediterranei*.



Scheme 21. Results of the feeding experiment with 3-azido-2-hydroxybenzoic acid (**63**) as a mutasynthon.

The bioreduction of aryl azides by *S. hygroscopicus* has been reported for the mutasynthons with the same 1,3,5-substitution pattern as in AHBA (**7**). After feeding with 3-azido-2-hydroxybenzoic acid (**63**) to the AHBA(−) mutant of *A. mediterranei*, no tetraketide was formed probably due to the fact that 2,3-disubstituted benzoic acids are not accepted by the loading domain (Scheme 21). Simultaneously, hydroxylation at C-5 followed by oxidation and a Michael addition to form a dihydronaphthohydroquinone did not take place after feeding with the mutasynthon **63**. However, the reduction product **103**, which was also detected after supplementation of 3-amino-2-hydroxybenzoic acid (**61**), was isolated in the amount of 11.1 mg/L (Figure 14).

Fermentations in the presence of the azido and nitro analogues of the natural starter unit AHBA provided rifamycins as well as pyranones and detoxification products with reduced nitro functionality. The reduction of the azido group may imply the presence of a specifically active azido reductase in *A. mediterranei* as it was also suggested for another strain explored in the Kirschning group – namely *Actinosynnema pretiosum*.[57] As a continuation of this research a series of chemical reactions was carried out using 3-azido-5-hydroxybenzoic acid (**94**) as a substrate to rule out a possible reduction of the azido group by free reducing agents (Table 3). The reactions were tested in different solvents (DMSO/H_2O, CH_3CN, CH_2Cl_2), buffers or their mixtures, in the presence of NADH as a reducing agent at 28 °C for 24 h, either in the presence or absence of magnesium perchlorate.

In case no product was observed, the reaction was continued for another 24 h stirring at 40 °C. None of these reactions provided 3-amino-5-hydroxybenzoic acid (**7**) and the substrate could still be detected in the reaction mixture.

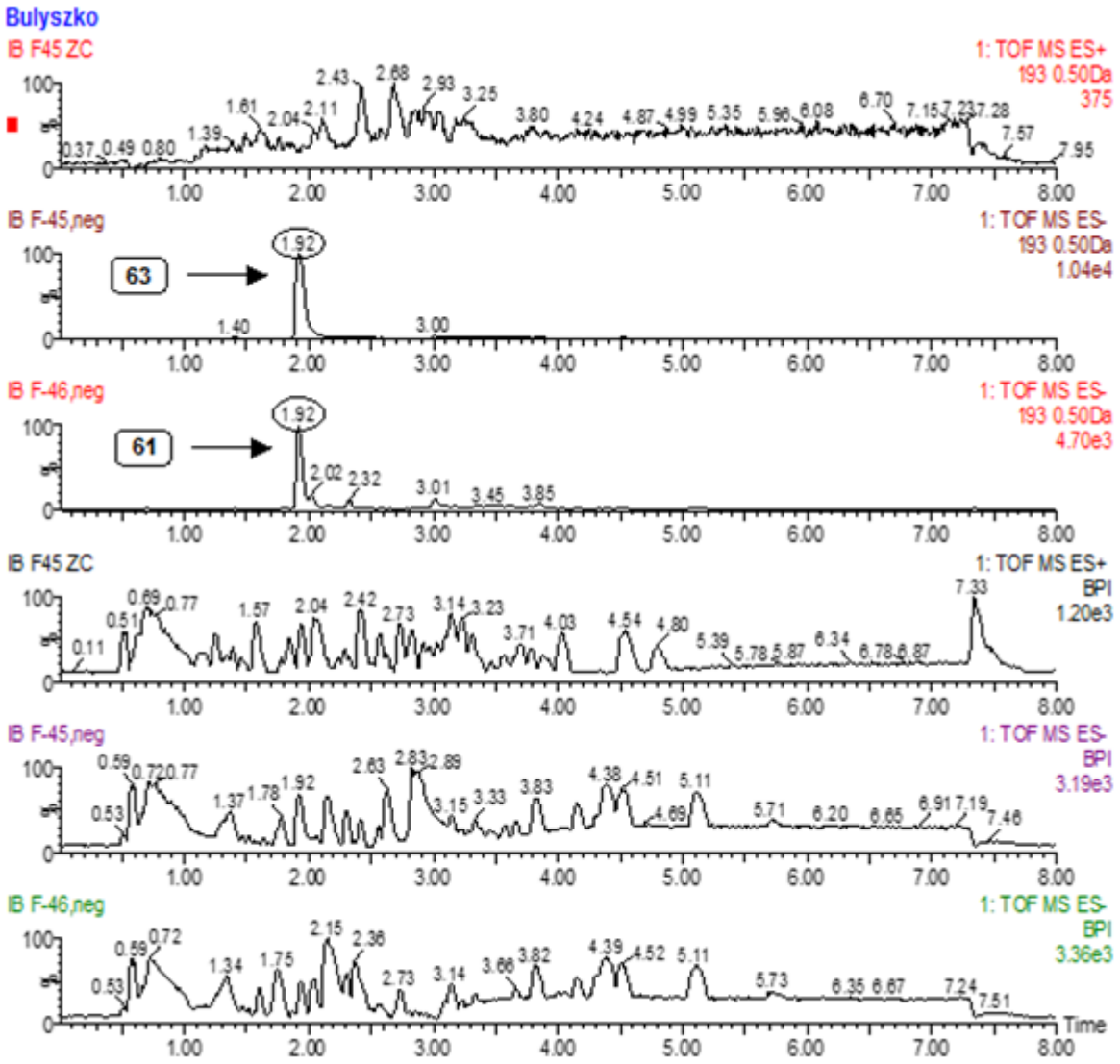
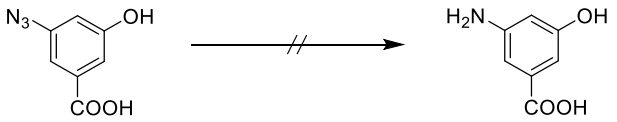


Figure 14. Chromatograms of the crude fermentation extracts after feeding with 3-azido-2-hydroxybenzoic acid (**63**, IB F-45) and 3-amino-2-hydroxybenzoic acid (**61**, IB F-46): $[M-H]^-$ = 193; t_R = 1.92 min. IB F45 ZC = zero control.



94 **7**

Entry	Substrate	NADH	$Mg(ClO_4)_2$	Solvent/Buffer	Conditions
1	1.0 eq.	1.5 eq.	x	phosphate buffer[a]	28 °C, 24 h 40 °C, 24h
2	1.0 eq.	1.5 eq.	x	succinate buffer[b]	28 °C, 24 h 40 °C, 24h
3	1.0 eq.	1.5 eq.	x	DMSO/H_2O (1:1)	28 °C, 24 h 40 °C, 24h
4	1.0 eq.	1.5 eq.	x	DMSO/H_2O + phosphate buffer[a]	28 °C, 24 40 °C, 24h
5	1.0 eq.	1.5 eq.	x	CH_3CN	28 °C, 24 h 40 °C, 24h
6	1.0 eq.	1.5 eq.	x	CH_2Cl_2	28 °C, 24 h 40 °C, 24 h
7	1.0 eq.	1.5 eq.	1.0 eq.	phosphate buffer[a]	28 °C, 24 h
8	1.0 eq.	1.5 eq.	1.0 eq.	succinate buffer[b]	28 °C, 24 h
9	1.0 eq.	1.5 eq.	1.0 eq.	DMSO/H_2O (1:1)	28 °C, 24 h
10	1.0 eq.	1.5 eq.	1.0 eq.	DMSO/H_2O + phosphate buffer[a]	28 °C, 24 h
11	1.0 eq.	1.5 eq.	1.0 eq.	CH_3CN	28 °C, 24 h
12	1.0 eq.	1.5 eq.	1.0 eq.	CH_2Cl_2	28 °C, 24 h

Table 3. Studies on the bioreduction of the azido group ([a] pH = 7, [b] pH = 4).

1.3.7 Biological Activity of Tetraketides

Biological tests were carried out in the Helmholtz Centre for Infection Research in Braunschweig, Germany. Tetraketides **41**, **80**, **94** and **98** have been analyzed in terms of their potential biological activity and cytotoxicity but all tests gave negative results (Table 4). The compounds submitted for the tests for the microbial activity with the use of three strains: *Escherichia coli*, *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) were found to be inactive and did not inhibit the growth of bacteria.

	Sample	*E. coli*			*P. aeruginosa*			MRSA		
		% growth			% growth			% growth		
		50µM	5µM	0.5µM	50µM	5µM	0.5µM	50µM	5µM	0.5µM
	98	105.0	112.4	105.0	115.7	108.1	109.9	76.1	92.9	286.2
	80	105.0	111.3	105.8	117.4	105.3	103.5	94.4	98.1	88.6
	41	104.2	111.8	104.3	108.0	104.6	100.9	98.6	81.9	96.0
	94	105.4	111.0	103.6	103.7	102.4	97.9	113.4	101.0	111.8
Ctrl 1		0.7	48.9	103.3	3.8	41.2	100.3	1.0	16.9	125.0
Ctrl 2		0.7	102.8	105.5	8.1	60.3	99.3	0.2	28.7	108.8
Ctrl 3		0.8	103.3	103.5	46.3	100.2	101.6	-0.3	66.5	142.2
DMSO		105.3	103.6	104.4	101.2	102.4	100.8	106.7	101.4	140.9
E. coli	Ciprofloxacin	20.0	2.0	0.10						
		10.0	1.0	0.10						
		5.0	0.5	0.05						
PA7	Amikacin				50.0	5.00	0.50			
					25.0	2.50	0.25			
					12.5	1.25	0.13			
MRSA	Linezolid							20.0	2.0	0.2
								10.0	1.0	0.1
								5.0	0.5	0.05

Table 4. The results of the tests for potential microbial activity with the use of mutasynthons **41**, **80**, **94** and **98.**

1.4 Summary and Outlook

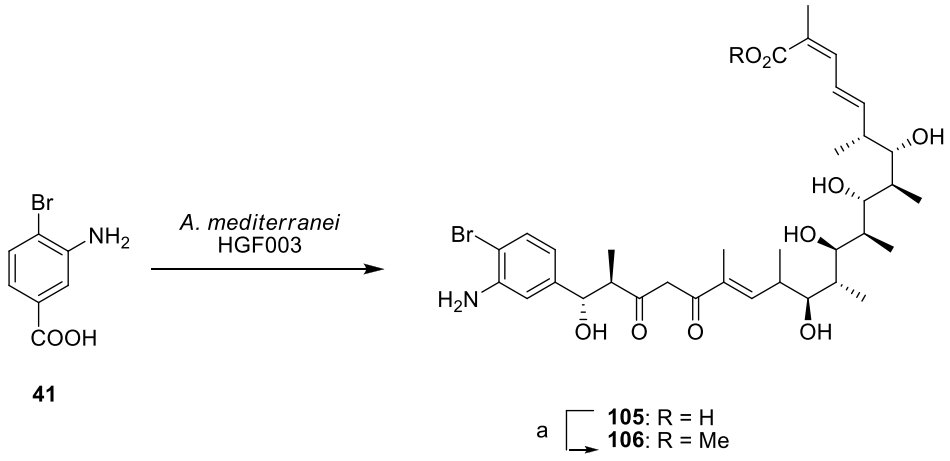
1.4.1 Summary and Conclusions

In conclusion, the synthetic potential of the blocked mutant of *A. mediterranei* HGF003, the producer of rifamycin B, was evaluated using the concept of mutasynthesis. The rifamycin loading domain, similarly to the loading domain of geldanamycin and ansamycin PKSs, was found to be highly tolerant to unnatural benzoic acids, especially to 3-aminobenzoic acids with an additional substituent located at position C-5. Simultaneously, high substrate specificity of the enzymes involved in the naphthalene formation has been revealed as any oxidation products formed from the employed mutasynthons could be detected.

Overall, thirty variously substituted and functionalized benzoic acids were fed to the AHBA(−) mutant strain of *A. mediterranei* HGF003 resulting in the isolation of thirteen tetraketides. A few of them were analyzed in terms of their potential biological activity and cytotoxicity but all tests gave negative results. Additionally, studies with azido- and nitro analogues of AHBA established remarkable reductive properties of *A. mediterranei* towards aryl azides and nitroarenes. It has been the second example of a microbial source possessing reductive properties towards aryl azide reported so far in the literature. As observed in *S. hygroscopicus*, the reduction likely takes place prior to loading onto the PKS and is not associated with the PKS enzymes. Unfortunately, the identification of the cryptic enzyme responsible for the reduction has not yet been achieved.[83,84]

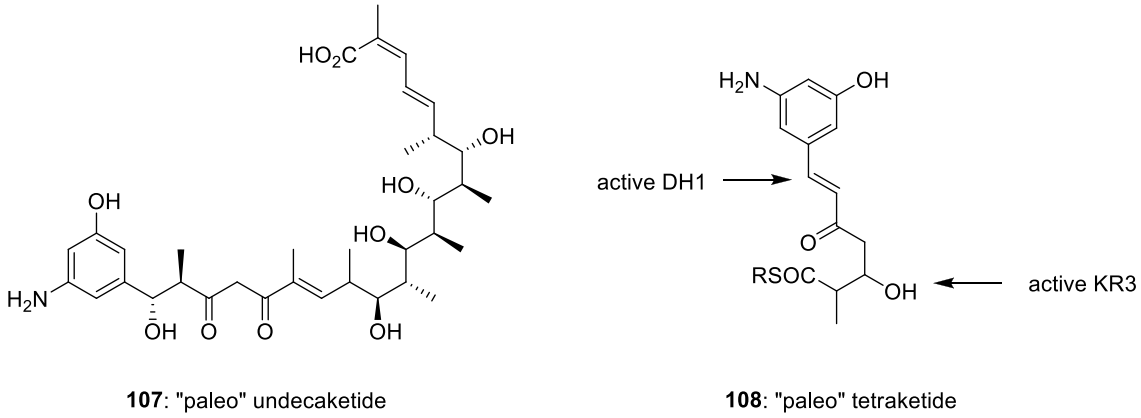
Feeding with 3-amino-4-bromobenzoic acid (**41**) revealed the formation of the undecaketide **105** which was detected in the crude fermentation extract by UPLC-HRMS (Scheme 22). Although the methyl ester **106** has been prepared from a crude fermentation product, attempts to isolate the undecaketide by HPLC have not been successful, most likely due to the chemical lability of the enedione structure in both compounds. The formation of the compound **105** is remarkable because it reveals that the *rif*-PKS is able to process the tetraketide which cannot be cyclized to the dihydronaphthoquinone moiety. In case of all the other mutasynthons fed to the mutant strain, failure of the ring closure resulted in release of the pyranone. Furthermore, the hypothetical “paleo” undecaketide **107** may be considered as an evolutionary predecessor of an unknown *rif*-PKS product as some evolutionary changes are evident in the *rif*-PKS organization – these are inactive DH from the module 1 and KR from the module 3 (Scheme 23). Inactivation of these two domains might have been crucial

for the formation of a dihydronaphthoquinone moiety especially that the hypothetical “paleo” tetraketide **108** does not possess any structural features to initiate cyclization.



Scheme 22. Detection of the undecaketide **105** in the crude fermentation material by UPLC-HRMS (t_R = 3.59 min; *m/z:* $[M+H]^+$ calcd for $[C_{36}H_{55}BrNO_9]^+$: 724.3060, found: 724.3058) and preparation of the methyl ester **106** for analytical purposes. Reaction conditions: a) $(CH_3)_3SiCHN_2$, MeOH/toluene, RT, 30 min.

As a result of evolution, new enzymes responsible for the dihydronaphthoquinone formation could become a part of the rifamycin biosynthesis. In fact, the meaning of evolutionary analyses has been discussed by Kalesse and co-workers in a report on the synthesis and biological evaluation of “paleo” soraphens which displayed a different biological activity than natural soraphen and affected other biological targets.[85,86]



Scheme 23. Structures of hypothetical „paleo” undecaketide **107** and „paleo” tetraketide **108**.

1.4.2 Outlook of the Project

Although a few tetraketides submitted for biological tests did not display any microbial activity, the remaining ones should be screened in terms of their potential biological activity and cytotoxicity. Additionally, some of the isolated 2*H*-pyran-2-one derivatives can be used as chiral building blocks and serve as synthetic tools to create libraries of more advanced intermediates. Biologically active and therapeutically important 2-pyranones, including 4-hydroxy-2-pyranone (or α,β-unsaturated lactones) have been found in a wide variety of organisms.[87–93] Furthermore, numerous 2-pyranones have been already used as precursors in the synthesis of pharmacologically important compounds such as anticancer agents, HIV protease inhibitors, antimicrobials, antifungals, cardiotonics and plant growth regulators.[94]

The high substrate tolerance of the loading domain of the *rif*-PKS for unnatural benzoic acids may be employed in the production of natural products derivatives through protein engineering techniques. While the benzoic acids were used as putative substrates in the project, the tolerance of the loading domain towards other types of aromatic compounds, e.g. variously substituted heterocyclic substrates, could be tested. Simultaneously, the identification of the cryptic enzyme responsible for the reduction of the nitro- and azido groups should be further deeply investigated.

Furthermore, fermentation with the use of 3-amino-4-bromobenzoic acid (**41**) should be repeated on a larger, few-liter scale, followed by methylation and acylation of the hydroxyl groups to enable isolation of the undecaketide in amounts sufficient for full characterization.

2 Geldanamycin

2.1 Introduction

Heat shock proteins belong to a family of molecular chaperones which display the highest level of expression among all cellular proteins across all species.[95–104] The heat shock protein 90 (Hsp90) is one of the most abundant eukaryotic heat-related proteins but it has also been reported in procariotic organisms.[105] There are two major forms of this protein in cytoplasm: the Hsp90α which is responsible for a fast cellular response and the Hsp90β which plays role in long-term adaptation of the cell.[99,106] The Hsp90 stabilizes many proteins (called client proteins) required for the tumor growth by assistance in their proper folding and maturation, as well as the regulation of the cell cycle. In 2000 Hanahan and Weinberg published six properties of Hsp90 client proteins which are necessary for the formation of a cancer cell from a normal cell that require many alterations in cell functioning (Figure 15).[107] These hallmarks are a) evasion of apoptosis, b) self-sufficiency in growth process, c) unlimited replication ability, d) insensitivity to anti-growth signals, e) ability to tissue invasion and metastasis, and f) sustained neoangiogenesis. Increased Hsp90 activity can often indicate the presence of cancer cells in a body.

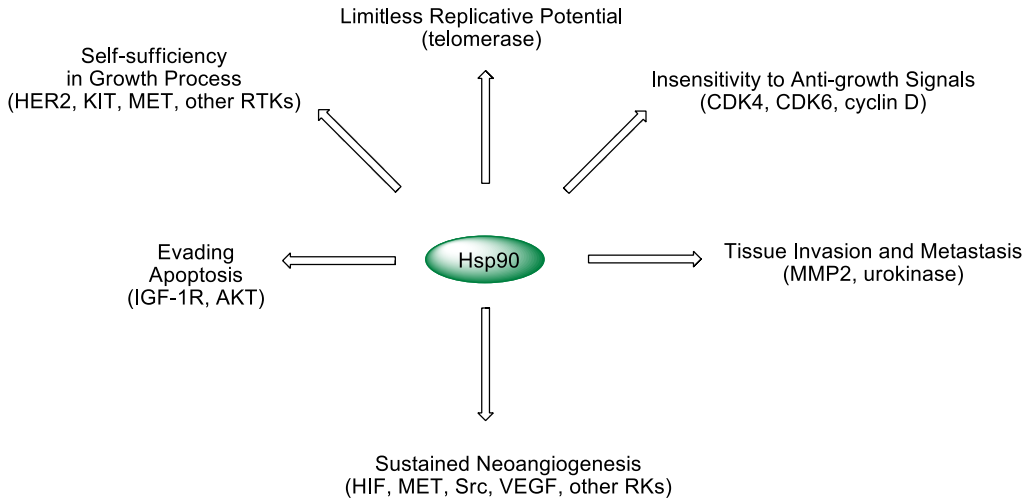


Figure 15. Hsp90 client proteins and six properties necessary for the formation of a cancer cell (RTK = receptor tyrosine kinase; IGF-1R = insuline-like growth factor; AKT = protein kinase B; HER2 = human epidermal growth factor receptor 2 (receptor tyrosine-protein kinase); KIT = cell growth factor receptor; MET = hepatocyte growth factor receptor; CDK = cyclin-dependent kinase; MMP2 = matrixmetalloproteinase-2; HIF = hypoxia-inducible factor; Src = proto-oncogene tyrosine-protein kinase; VEGF = vascular endothelial growth factor).

A significant number of natural products have been identified as inhibitors of the chaperone function of Hsp90 so far.[108] Among the most famous are geldanamycin (**4**), herbimycin (**109**)[109,110] and macbecin (**110**)[111] which belong to the group of ansamycin antibiotics (Figure 16).

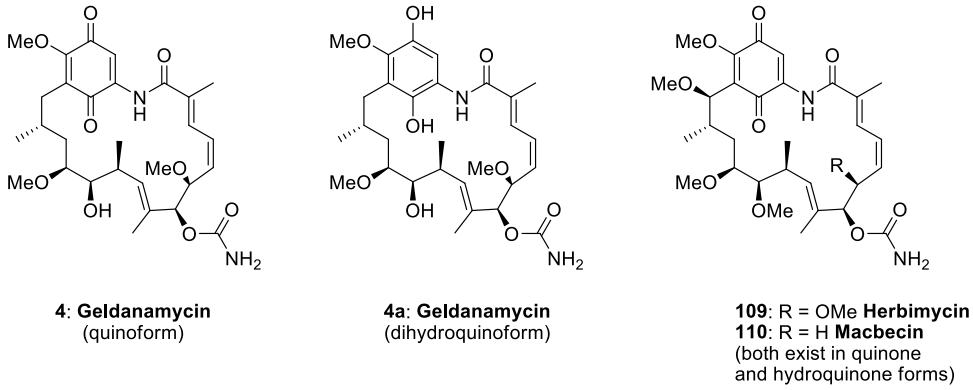


Figure 16. Chemical structures of ansamycin-class Hsp90 inhibitors.

2.1.1 Biological Activity of Geldanamycin and Its Derivatives

Geldanamycin (**4**) is a benzoquinone ansamycin antibiotic first isolated in 1970 from a culture extract of *Streptomyces hygroscopicus* var. *geldanus* var. *nova*.[112] It displays antitumor properties by binding to the *N*-terminal ATP-binding pocket of Hsp90 and inhibition of its ATP-dependent chaperone activity which initiates the release of client proteins and their degradation by the ubiquitin-proteasome pathway.[113]

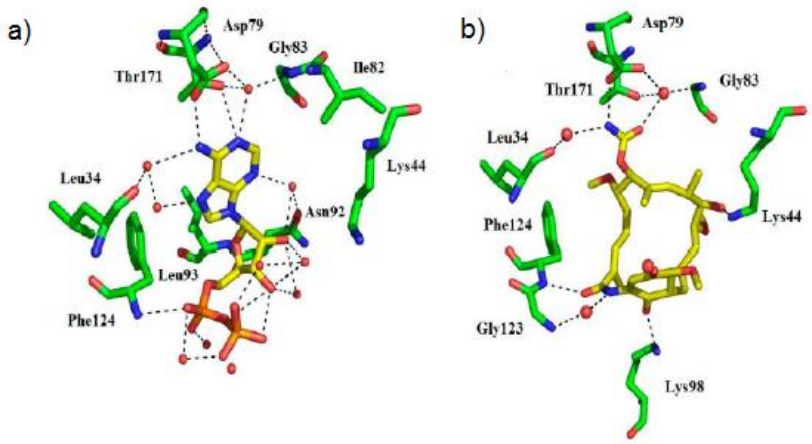


Figure 17. Crystal structures of a) ADP and b) geldanamycin co-crystallized with yeast Hsp90.[114]

As it has been proven by the crystal structure of geldanamycin co-crystallized with the yeast Hsp90 (Figure 17), the macrocyclic ring and the carbamate group at C-7 of the compound **4**

are directed towards the bottom part of the binding pocket, while the benzoquinone ring is oriented towards the top of the pocket.[115] Thus, the benzoquinone moiety interacts similarly to the phosphate backbone of ATP/ADP, while the carbamate group imitates H-bonding interactions of the purine system of ATP/ADP.

Despite its anticancer *in vitro* potential in a wide range of cancers, geldanamycin has not been considered for clinical use due to a number of limitations: metabolic instability, severe hepatotoxic activity and low solubility in aqueous solutions.[116,117] Instead, a few analogues of geldanamycin have been developed in semisynthetic approaches, mainly by alterations in the quinone structure at the C-17 postion and became drug candidates at different stages of clinical trials. Among them, tanespimycin (17-AAG, **111a**) and alvespimycin (17-DMAG, **112**) have been the most promising (Figure 18).[118–121]

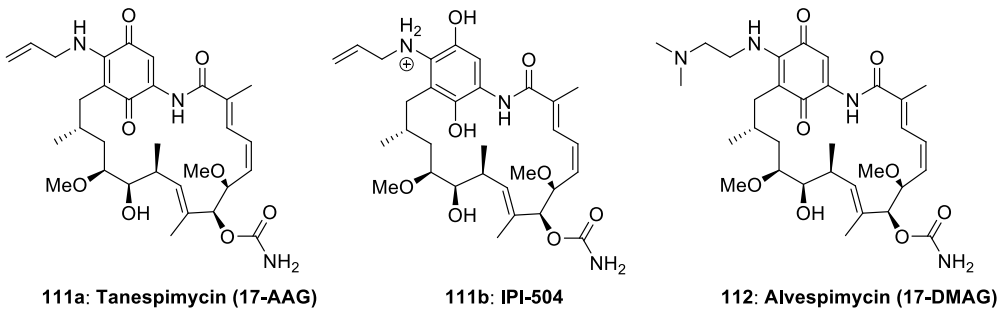


Figure 18. Structures of geldanamycin derivatives - potential drug candidates.

17-AAG (**111a**) has displayed anticancer properties at low nanomolar concentrations and entered clinical trials to evaluate its activity in patients with melanoma, prostate or breast cancer.[117] The proposed mechanism of action of 17-AAG involves downregulation of some oncoproteins and reduction of the cell growth. Unfortunately, the compound **111a** exhibited poor solubility and limited oral bioavailability. However, the latter case can be modulated by administration with other drugs, e.g. cyclosporine. 17-DMAG was found to be more active on tumor explants than 17-AAG but is less metabolized.[119]

Additionally, some new fluorogeldanamycin derivatives **113**-**117** were obtained in a mutasynthetic approach by Kirschning and co-workers after feeding with monofluoro 3-aminobenzoic acids to an AHBA-blocked mutant of *S. hygroscopicus* (Figure 19).[54] Although the compounds **113**-**117** are devoid of either quinone or hydroquinone moiety, they display very strong antiproliferative properties towards cancer cell lines. The geldanamycin derivative **113** is expected to be perfectly suited for further clinical evaluations. Furthermore,

two novel geldanamycin analogues **118** and **119** with higher solubility in water and decreased cytotoxicity were discovered in 2013 in *S. hygroscopicus* 17997.[122] However, in this case more studies have to be conducted for the evaluation of these compounds as anticancer drug candidates. The strain *S. hygroscopicus* 17997 has also been found to produce sulfur-containing geldanamycin derivatives **120**-**121** as reported by Wang in 2011.[123,124]

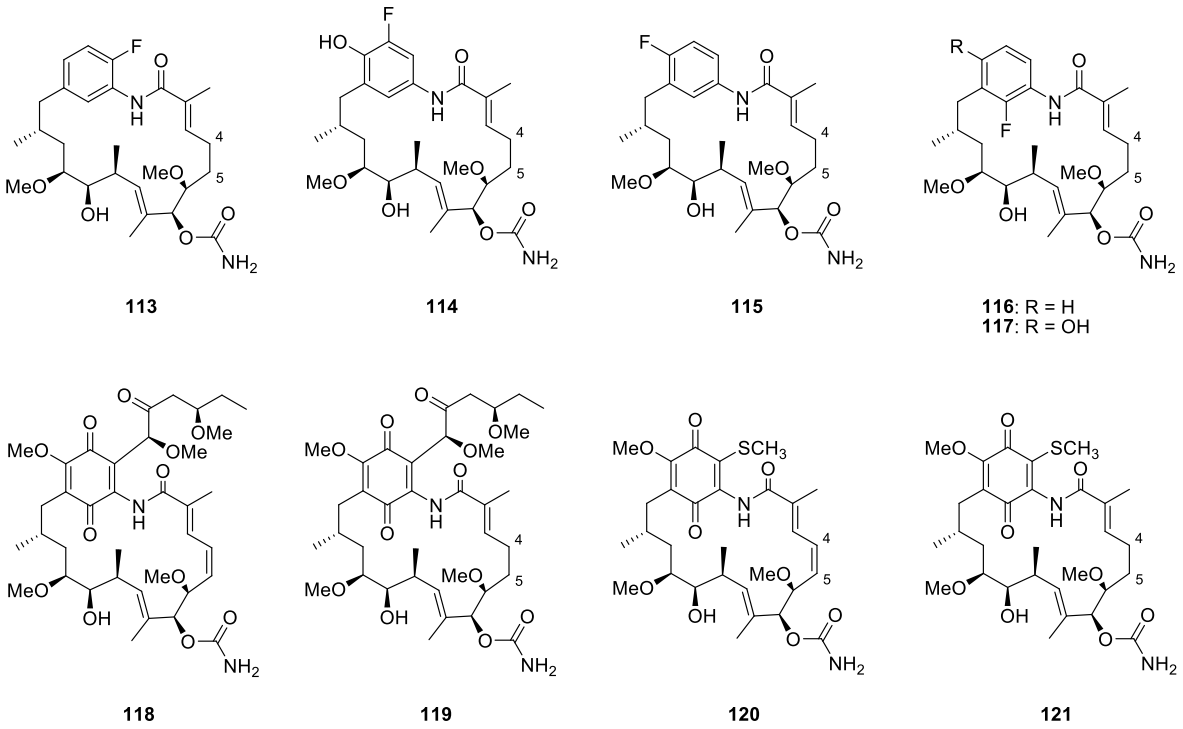
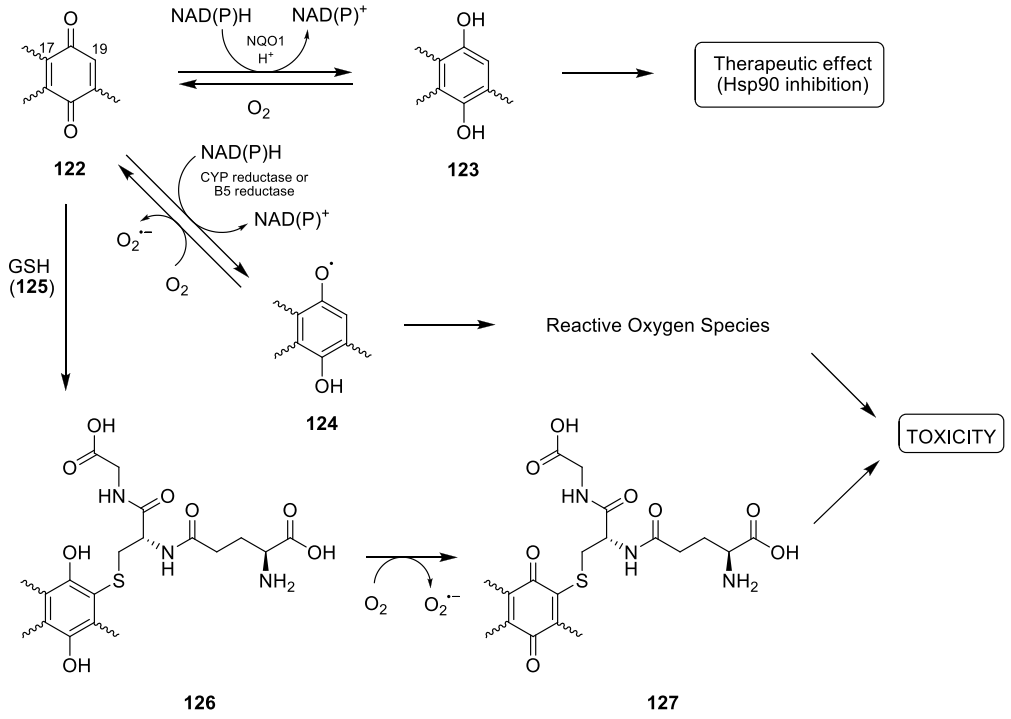


Figure 19. Some geldanamycin derivatives as promising anticancer drug candidates.

The toxicity of geldanamycin is caused by the metabolism of the benzoquinone moiety. This benzoquinone toxicophore **122** undergoes a two-electron reduction involving NAD(P)H/quinone oxidoreductase 1 (NQO1) to the dihydroquinones **123** which display higher Hsp90 inhibitor activity and better solubility in water. The benzoquinones **122** can also be metabolized with one-electron reductases: CYP reductase and B5 reductase generating the unstable semiquinones **124** which can be further oxidized to superoxide radicals by molecular oxygen (Scheme 24).[125] Furthermore, as suggested by Strong, sulfur-containing nucleophiles, e.g. glutathione (GSH, **125**), are able to modulate the toxicity of the quinone compounds. Benzoquinones **122** can interact with GSH via a classic 1,4-reductive Michael addition providing the hydroquinone conjugate intermediates **126** which are oxidized to the quinone conjugates **127** and thus inducing toxicity.[126]

As benzoquinone is an unfavorable structural feature of Hsp90 inhibitors, many approaches have been developed to modify the quinone moiety. In 2008 Martin and co-workers reported the use of biosynthetic engineering to deactivate the monooxygenase responsible for the quinone formation leading to the optimization of Hsp90 inhibitor macbecin (**110**).[127] Additionally, small synthetic molecules, e.g. SNX-2112, active as Hsp90 inhibitors and devoid of the quinone moiety have been obtained.[128]



Scheme 24. A proposed metabolic pathway of benzoquinones by one- and two-electron reductases and conjugation with glutathione.

2.1.2 Structure-Activity Relationship in Geldanamycin

Ansamycin antibiotics are unique natural products not only because of their biological activity but also from a synthetic point of view. In the structure-activity studies summarized in a review[95] by Kirschning and co-workers three tactics have been employed: synthetic, semisynthetic and mutasynthetic. Among all the ansamycins tested, over 500 geldanamycin derivatives have been analyzed and as a conclusion of these studies the ”SAR map” has been proposed (Figure 20).

It was shown that introduction of a wide range of modifications was possible in the structure of geldanamycin. Modifications in the part spreading from C8 to C15 as well as the substitution pattern on the aromatic ring did not have a significant impact on the biological

activity of the compound **4** and its derivatives. However, the presence of a carbamate at C-7 has been proved to be essential for the biological properties as it interacts with the carboxyl group of Asp79 in the Hsp90 binding pocket.[114,129,130] Additionally, alkylation of the lactam nitrogen atom and removal of the methyl- or the methoxy substituents at C-6 results in loss of toxicity.

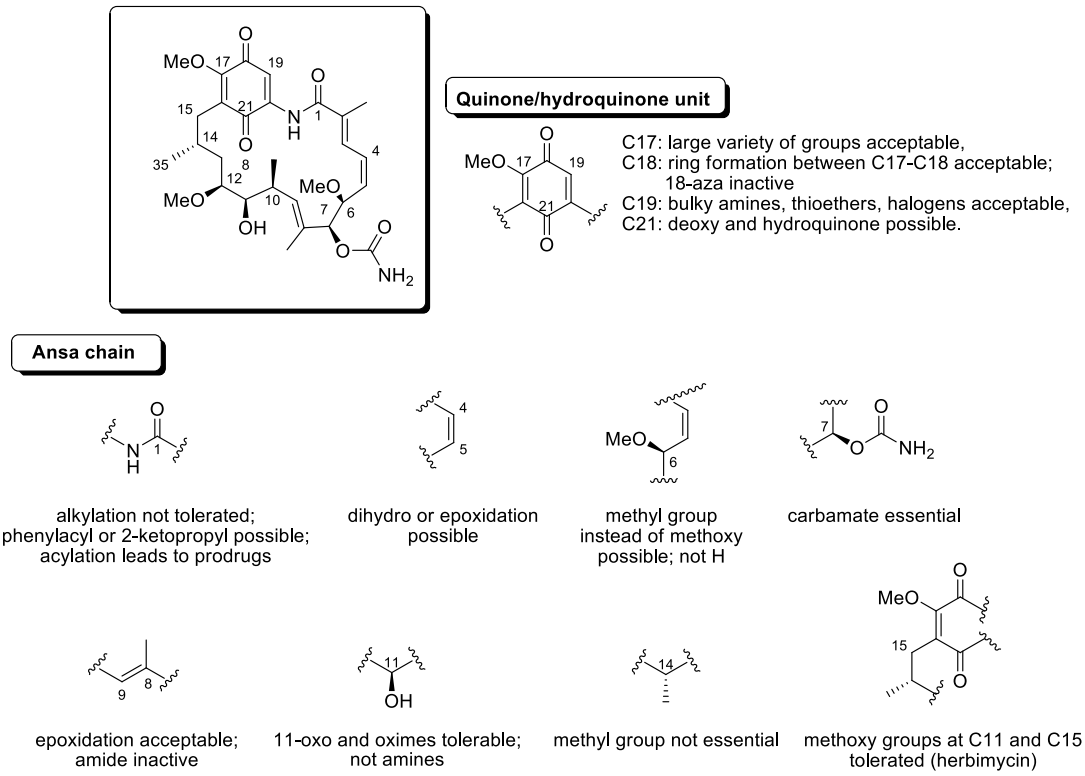
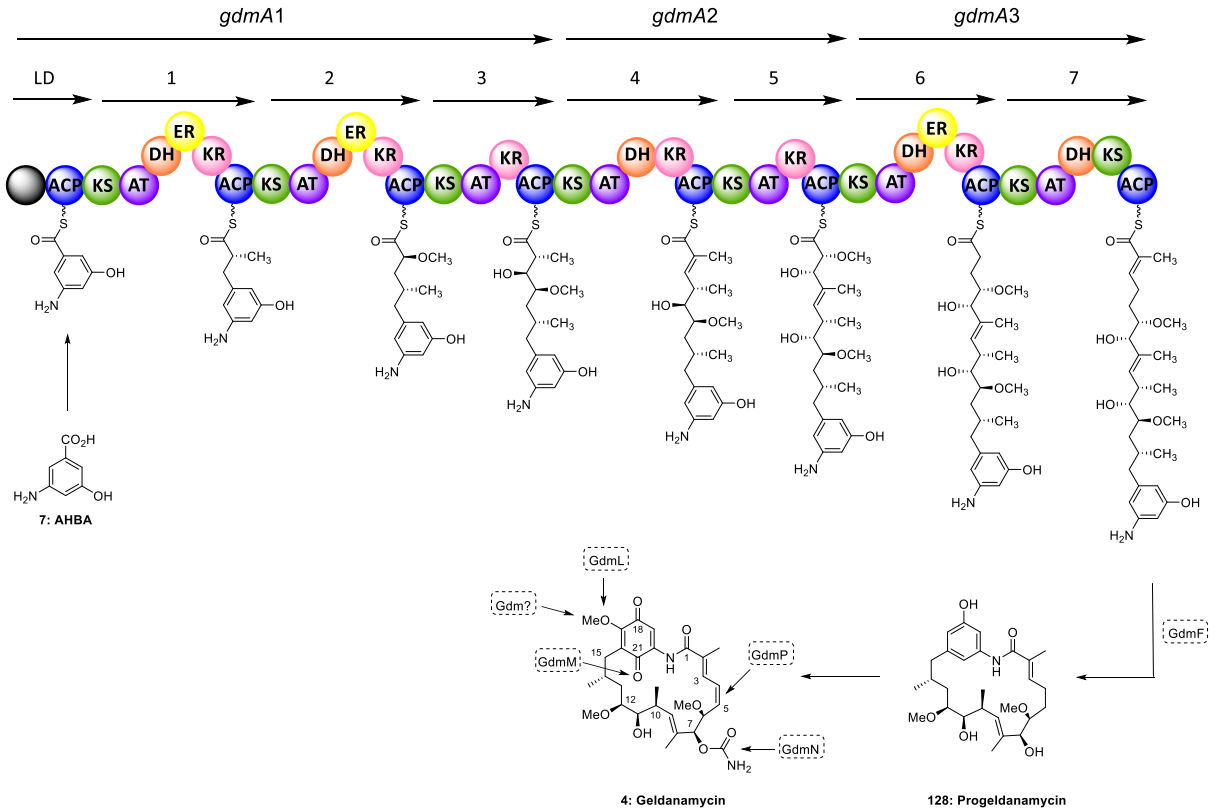


Figure 20. Conclusions of structure-activity studies in geldanamycin and its derivatives.[95]

2.1.3 Geldanamycin Biosynthesis

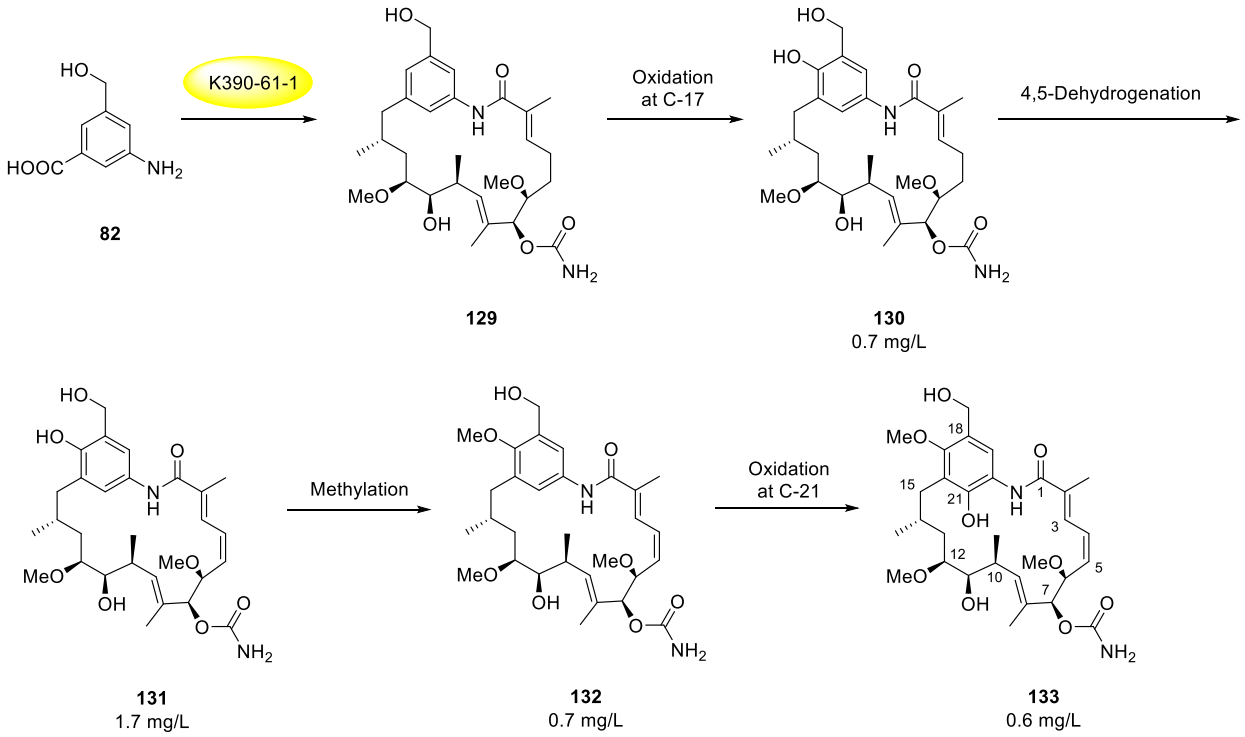
The biosynthesis of geldanamycin (**4**) has been proposed by the Hutchinson group and it is presented in Scheme 25.[131,132] The compound **4** is biosynthesized by the seven-module type I PKS, encoded by the *gdm*A1-A3 genes, from AHBA (**7**) and chain extender units: malonyl-CoA, methylmalonyl-CoA and 2-methoxymalonyl-CoA. As in the case of rifamycins, the linear product released from the *gdm*-PKS is cyclized by the amide synthase encoded by *gdm*F gene to give progeldanamycin (**128**). The macrolactam product **128** is then subjected to the post-PKS transformations by tailoring enzymes: oxidation at C-17 (GdmL), oxidation at C-21 (GdmM), 4,5-dehydrogenation (GdmP), carbamoylation at C-7 (GdmN) and *O*-methylation at C-17 (enzyme not known). The post-PKS modifications have been investigated in 2004 by Hong and co-workers[133] and a few years later by the Kirschning

group who proposed a sequence of post-PKS steps on the basis of mutasynthetic feeding experiments with AHBA(−) blocked mutant of *S. hygroscopicus* K390-61-1 (Scheme 26).[134]



Scheme 25. The geldanamycin biosynthesis in *Streptomyces hygroscopicus* (LD = loading domain, ACP = acyl carrier protein; KS = ketosynthase; AT = acyltransferase; DH = dehydratase; ER = enoylreductase; KR = ketoreductase).

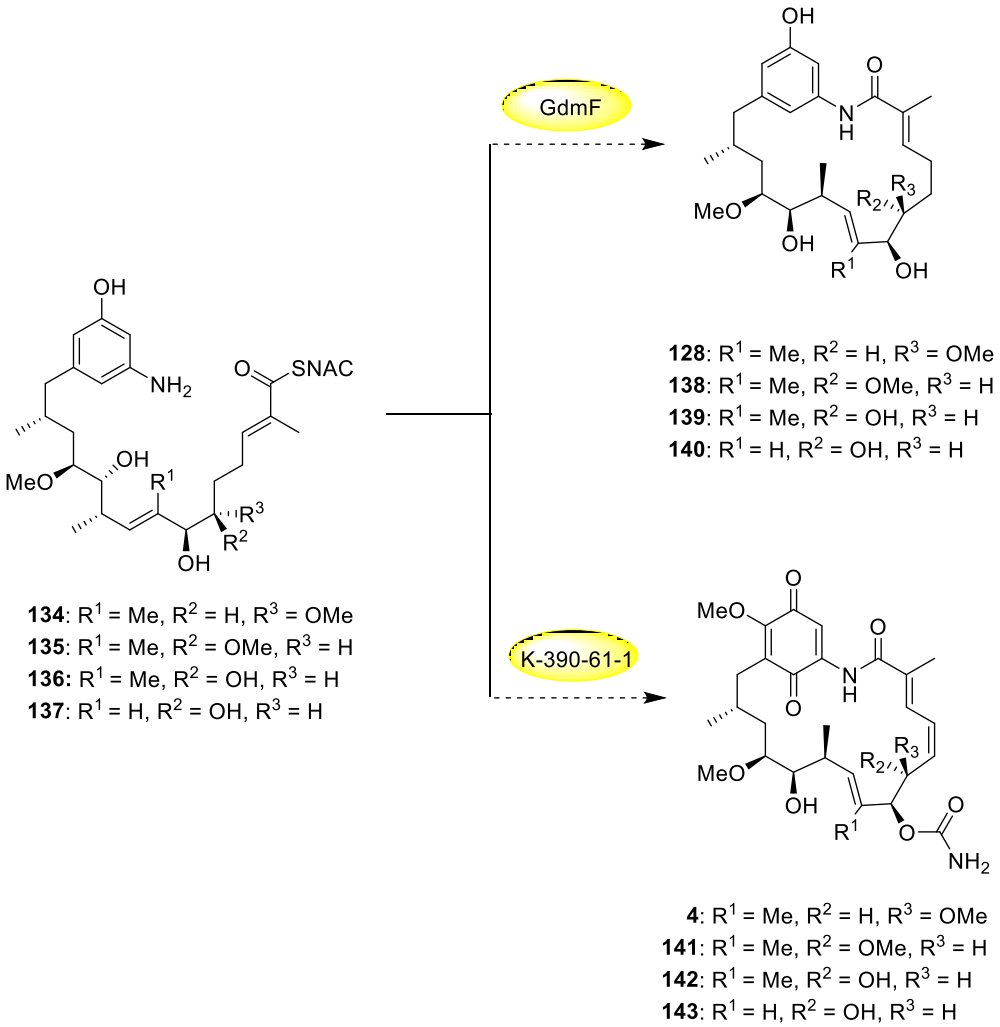
Kirschning and co-workers employed 3-amino-(5-hydroxymethyl)benzoic acid (**82**) as a mutasynthon processed to a variety of geldanamycin derivatives by a mutant strain of *S. hygroscopicus* which were isolated and fully characterized. Carbamoylation at C-7 which yielded the compound **129** was proposed as the initiating step of the post-PKS modifications. The mutaproduct **129** was then oxidized at C-17 to provide the phenol **130**, followed by a 4,5-dehydrogenation step and the formation of the compound **131**. The last two transformations were *O*-methylation at C-17 providing the mutaproduct **132** and oxidation at C-21 after which the fully transformed product **133** was obtained. The proposed sequence of modifications differs from the one proposed by Hong[133] who placed carbamoylation at a later stage of the geldanamycin biosynthesis.



Scheme 26. Mutasynthetic feeding experiments with the benzoic acid **82**.

2.2 Purpose of the Project: Synthesis of Progeldanamycin Derivatives

The purpose of this project is the investigation of the tolerance and biosynthetic transformation of the complex substrates **134-137** by the heterologously expressed amide synthase (GdmF), the enzyme responsible for the macrolactamization in the biosynthesis of geldanamycin. To this end, the *seco*-progeldanamycin acid derivatives **134-137** are planned to be synthesized by total synthesis and used in enzyme expression studies of the amide synthase in which new progeldanamycin derivatives **138-140** are expected to be formed. Additionally, the SNAc esters could be fed to a knockout strain of *S. hygroscopicus var. geldanus* (K-390-61-1), which is incapable of performing the AHBA biosynthesis, to obtain new geldanamycin derivatives **141-144** (Scheme 27).

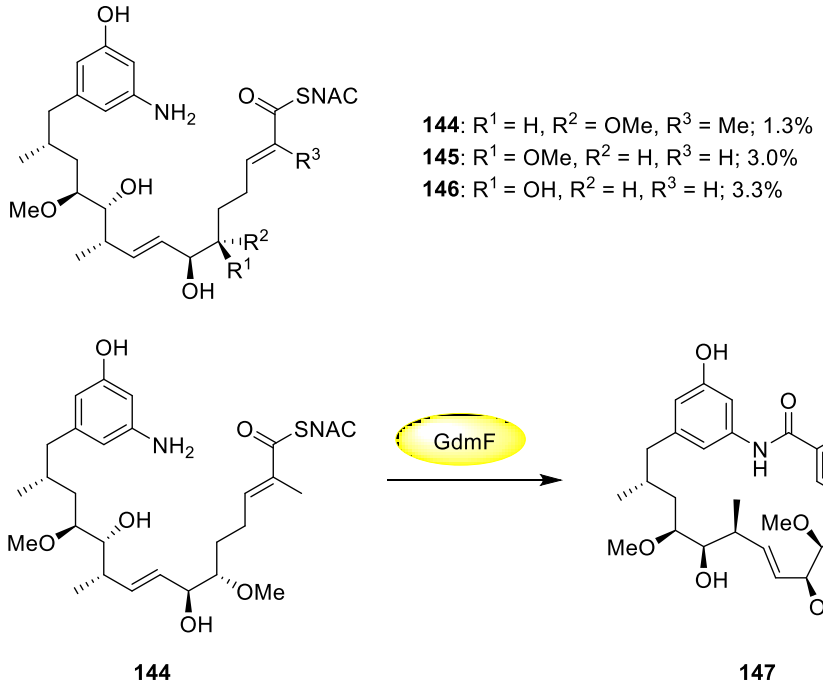


Scheme 27. Planned studies towards substrate flexibility of the amide synthase GdmF in enzyme assays and feeding experiments with the mutant of *S. hygroscopicus var. geldanus* K-390-61-1.

2.3 Discussion and Results

2.3.1 Previous Work on the Substrate Flexibility of the Amide Synthase

To investigate the substrate flexibility of the amide synthase both in enzyme assays and in the mutasynthetic approach with the mutant strain of *S. hygroscopicus var. geldanus*, Jekaterina Hermane prepared three SNAc esters **144**-**146**. Unfortunately, feeding experiments with the *seco*-progeldanamycin acid derivatives **144**-**146** did not provide satisfying information on the flexibility of the amide synthase, probably due to poor membrane permeability or instability of the synthesized substrates. However, the compound **144** was successfully used in the enzymatic reaction with the heterologously expressed amide synthase which resulted in the formation of a geldanamycin derivative **147** (Scheme 28).[135] This proved that GdmF is able to accept complex and modified biosynthetic intermediates and transform them to the corresponding macrolactams.



Scheme 28. Structures of *seco*-progeldanamycin derivatives **144**-**146** prepared by Jekaterina Hermane and enzymatic reaction with the SNAc ester **144** and the amide synthase.[135]

The preparation of the amide synthase GdmF and the optimization of the enzyme assays was developed in cooperation with Anja Heutling and Dr. Carsten Zeilinger. The synthesis of the *seco*-progeldanamycin acid derivatives is based on the previous work of Monika Vogt and Sascha Ceylan.[136,137]

2.3.2 Retrosynthesis

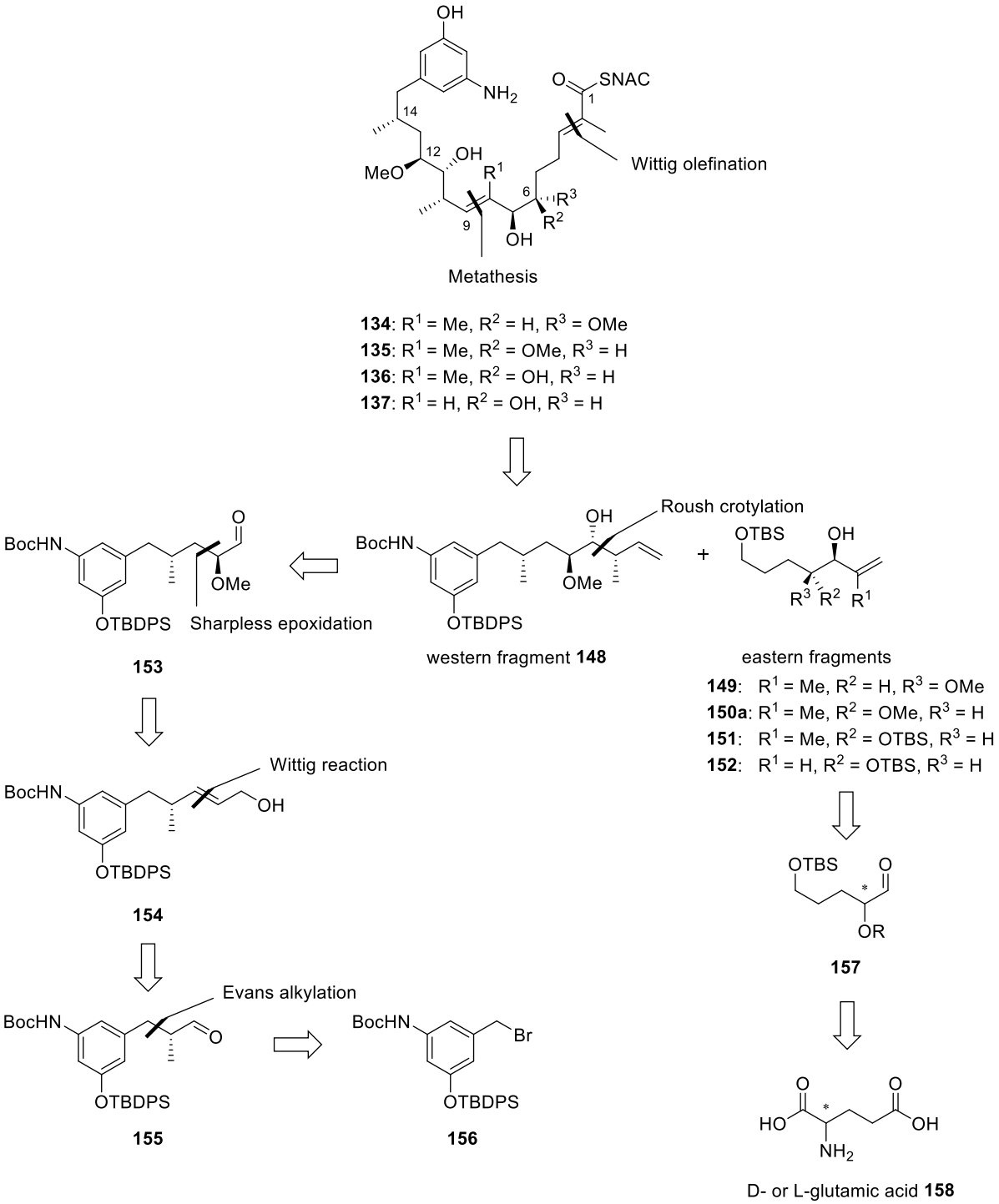
In the selection of the target SNAc esters synthesized by Jekaterina Hermane[135] as well as the compounds prepared in this thesis, the structure-activity relationship described in the section 2.1.2 has been taken into account. As the presence of the carbamate group at C-7 was proved to be essential for binding to the Hsp90, the hydroxyl function at C-7 should not be changed. Moreover, the substituents at C-6 play an important role as the removal of the methoxy group results in loss of toxicity. In contrast to this, modifications at C-8 should not have a significant impact on the anticancer activity of the compound.

As a result of these considerations, the *seco*-progeldanamycin acid derivatives **133**-**136** were chosen to be synthesized (Scheme 29). The compounds **133**-**136** will be prepared in their *N*-acetylcysteamine thioester forms (SNAc esters). The enzyme assays and feeding experiments with the substrates **133**-**136** should provide information about the influence of the methyl groups at C-2 and C-8 on the acceptance by the amide synthase and about the impact of a substituent at C-6 on the biological activity.

The planned strategy to synthesize the SNAc esters of the *seco*-progeldanamycin acid derivatives **133**-**136** has been presented in Scheme 29. As in the previous work carried out in the Kirschning group, each SNAc ester was retrosynthetically divided into two main parts: the western and eastern fragments, connected by means of olefin cross-metathesis.

The introduction of the SNAc ester will be performed using the Wittig olefination. The western fragment **148** should be prepared from the aldehyde **153** through the selective boron-mediated Roush crotylation. The synthesis of the compound **153** has been developed by Monika Vogt,[136] while the Roush crotylation has been optimized by Jekaterina Hermane.[135] The key steps in the synthesis of the aldehyde **153** are the Sharpless epoxidation followed by an epoxide ring opening to introduce a stereocenter at C-12, the Wittig reaction and the Evans alkylation to form a stereocenter at C-14.

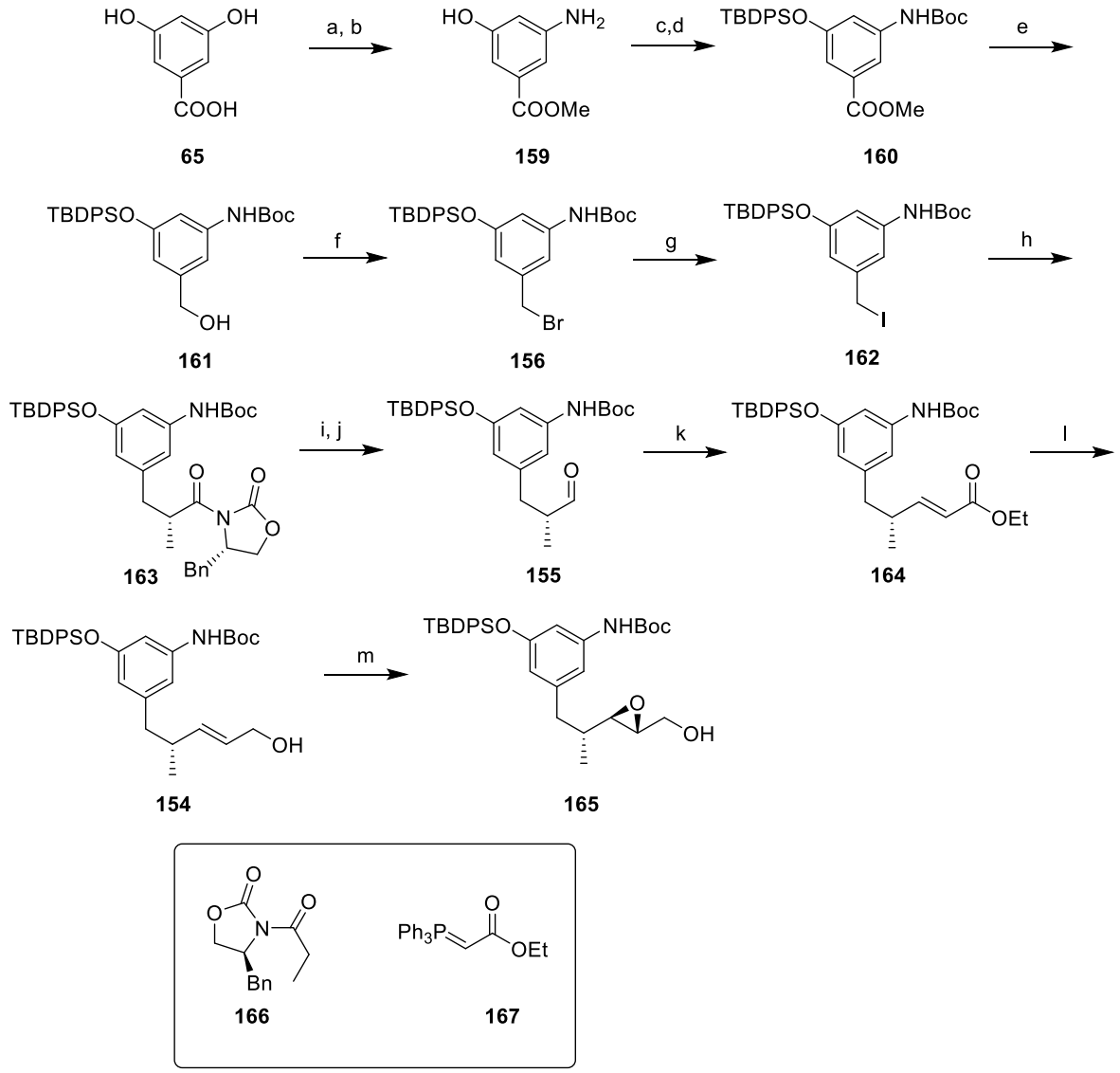
The formation of the double bond in the eastern fragments **149**-**152** will be carried out through either *syn*- or *anti*-selective Grignard addition from the aldehyde **157**. The synthesis of the corresponding aldehydes will be accomplished from the commercially available D- or L-glutamic acids (**158**-D and **158**-L), depending on the desired stereochemistry of the final product.



Scheme 29. Retrosynthetic analysis of SNAc esters **134-137**.

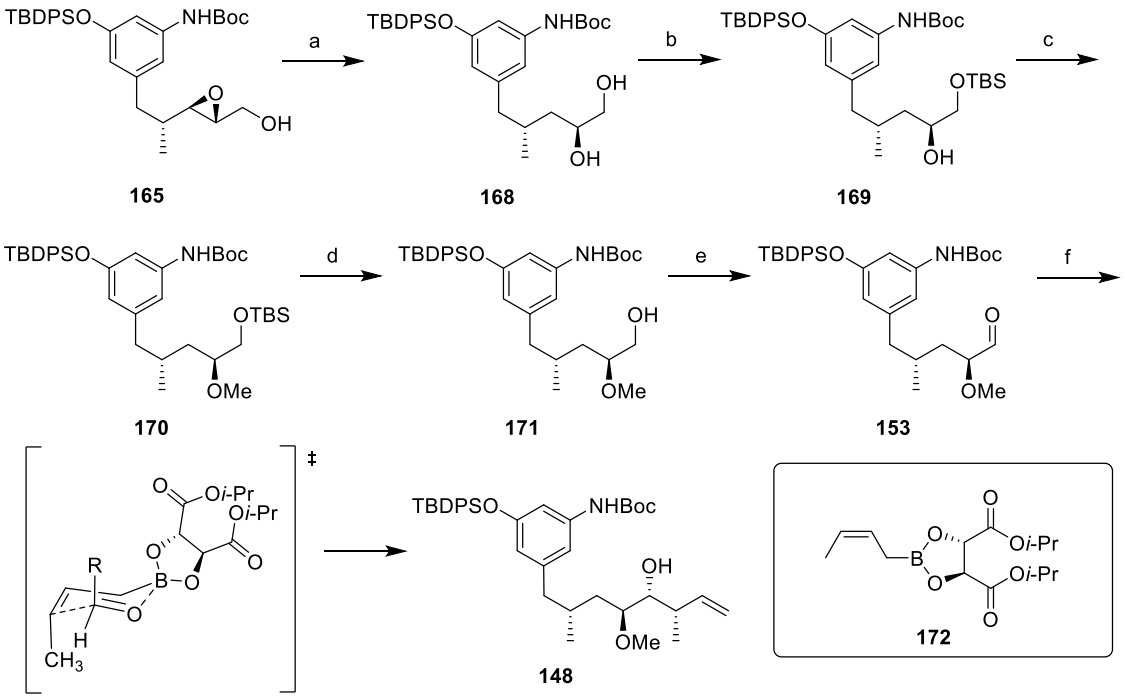
2.3.3 Synthesis of the Western Fragment

The synthesis of the western fragment **148** started from 3,5-dihydroxybenzoic acid (**65**). The preparation of the methyl ester **159** from the compound **65** was reported in the literature in 1983 by Rickards and involved a reaction with ammonia, followed by esterification with acetyl chloride in methanol (Scheme 30).[138]



Scheme 30. Synthesis of the epoxide **165**. Reaction conditions: a) conc. aq. NH_3, NH_4Cl, 180 °C, 40 h; b) acetyl chloride, MeOH, 65 °C, 36 h; c) Boc_2O, $NaHCO_3$/THF, RT, 44 h; d) TBDPSCl, imidazole, 4-DMAP, CH_2Cl_2, 30 °C, 16 h, 84%; e) DIBAL-H, THF, −78 °C → RT, 16 h, 94%; f) PPh_3, CBr_4, CH_2Cl_2, RT, 30 min, 99%; g) NaI, acetone, RT, 2 h, 95%; h) oxazolidinone (*S*)-**166**, LDA, THF, −35 °C, 13 h, 60%, d.r. > 10 : 1; i) $LiBH_4$, Et_2O, 0 °C, 1.5 h, 78%; j) DMP, $NaHCO_3$, CH_2Cl_2, RT, 1 h, 99%; k) **167**, $CHCl_3$, 50 °C, 15 h, 72%; l) DIBAL-H, CH_2Cl_2, −78 °C, 5 h, 75%; m) Ti(O*i*-Pr)$_4$, *t*-BuOOH, D-(−)-DET, MS 4Å, CH_2Cl_2, −20 °C, 20 h, 80%, d.r. > 10 : 1.

The further steps providing the benzyl alcohol **161** were established in the Kirschning group by Marco Brünjes and Florian Taft.[139–141] The Boc- and TBDPS-protection reactions of the ester **159** provided the compound **160** in 84% yield over the first four steps. Reduction of the ester **160** with diisobutylaluminium hydride to the benzyl alcohol **161** proceeded in 95% yield. Further synthesis towards the western fragment **148** was developed by Monika Vogt and Jekaterina Hermane.[135,136] The Appel reaction with triphenylphosphine and tetrabromomethane in CH_2Cl_2 provided the benzyl bromide **156** in 99% yield. The compound **156** was then subjected to the Filkenstein reaction affording the benzyl iodide **162** in 95% yield. The subsequent Evans alkylation with (*S*)-oxazolidinone **166** gave the desired diastereomer **163** in good yield. After removal of the auxiliary through reduction with lithium borohydride, the obtained alcohol was oxidized with the Dess-Martin periodinane to the aldehyde **155** which was then subjected to the Wittig reaction with triphenyl phosphonium ylide (**167**) providing the α,β-unsaturated ester **164** in 72% yield. The obtained ester **164** was reduced with DIBAL-H to the allyl alcohol **154** which reacted in the Sharpless epoxidation to give the desired epoxide **165** in 80% yield and a diastereomeric ratio higher than 10 : 1.



Scheme 31. Synthesis of the western fragment **148**. Reaction conditions: a) DIBAL-H, Et_2O, RT, 5 h, 85%; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 20 min, 74%; c) Proton Sponge, Me_3OBF_4, CH_2Cl_2, RT, 1 h, 85%; d) $LiBF_4$, MeCN, CH_2Cl_2, RT, 2 d, 75%; e) DMP, $NaHCO_3$, CH_2Cl_2, RT, 1 h, 99%; f) **172**, MS 4Å, toluene, −78 °C, 20 h, 80%, d.r. > 10 : 1.

In the second part of the synthesis, the epoxide **165** was reduced with DIBAL-H to give the diol **168** in 85% yield (Scheme 31). After the TBS-protection of the primary alcohol, methylation with trimethyloxonium tetrafluoroborate in the presence of Proton Sponge provided the compound **170** in 85% yield. The following deprotection and oxidation with DMP afforded the aldehyde **153** which was submitted to the last step of the western fragment synthesis – a stereoselective Roush crotylation. For the purpose of this reaction the (*S,S*)-crotylboronate **172** was prepared according to the procedure described in a total synthesis of reblastatin.[142] The synthesis of **172** involved two steps: preparation of the (*Z*)-crotylaminoboronate complex from *cis*-butene, triisopropyl borate and diethanolamine, followed by the reaction with (*S,S*)-diisopropyl tartrate. Finally, the crotylation reaction between the aldehyde **153** and the (*S,S*)-crotylboronate **172** provided the desired western fragment **148** in 80% yield and a diastereomeric ratio higher than 10 : 1. The traces of the undesired diastereomer could easily be separated by flash chromatography.

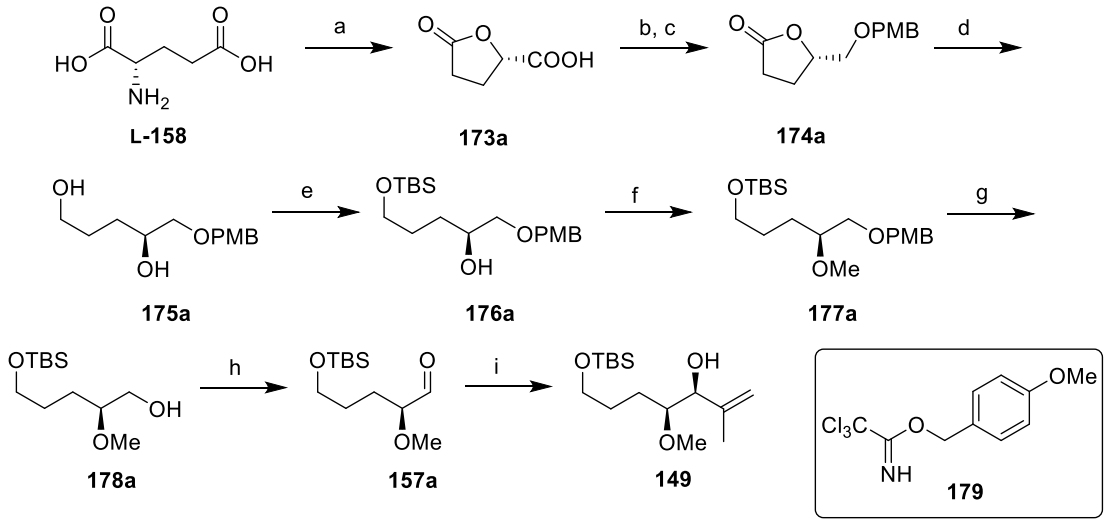
2.3.4 Synthesis of the Eastern Fragments

2.3.4.1 Synthesis of the Eastern Fragment 149

The synthesis of the eastern fragment **149** started with the diazotization of commercially available L-glutamic acid **(158**-L) providing the lactone **173a** in 53% yield (Scheme 32). The compound **173a** was then reduced with borane-dimethyl sulfide complex according to the procedure described by Herdeis, which afforded the corresponding hydroxymethyl derivative in 97% yield.[143] Subsequently, the hydroxyl group was protected using *p*-methoxybenzyl trichloroacetimidate (**179**) which was prepared from 4-methoxybenzyl alcohol and trichloroacetonitrile as described by Jacobsen.[144] In the next step the PMB-protected lactone **174a** was reduced to the diol **175a** in 78% yield using lithium aluminium hydride. The following TBS-protection of the primary alcohol and the methylation of the second hydroxyl group provided the compound **177a** in 94% yield. Removal of the *p*-methoxybenzyl group with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone afforded the alcohol **178a** in 89% yield, which was subsequently oxidized to the aldehyde **157a** with the DMP reagent.[135]

The key step in the synthesis of the eastern fragment **149** was the Grignard addition in which stereoselectivity had to be controlled. For the transformation of the aldehyde **157a** into the desired compound **149** two sets of conditions have been tested (Table 5). First, the carbonyl compound **157a** was treated with isopropenylmagnesium bromide at −78 °C in CH_2Cl_2 in the presence of magnesium bromide ethyl etherate as a Lewis acid.[145] This reaction provided the

desired *syn*-product **149** in 53% yield and with diastereomeric ratio *syn* : *anti* > 95 : 5. The second reaction was carried out in THF at −78 °C but without the Lewis acid leading to the product **149** in similar yield and with the same diastereoselectivity. Finally, the eastern fragment **149** has been prepared in 10% yield over nine steps.



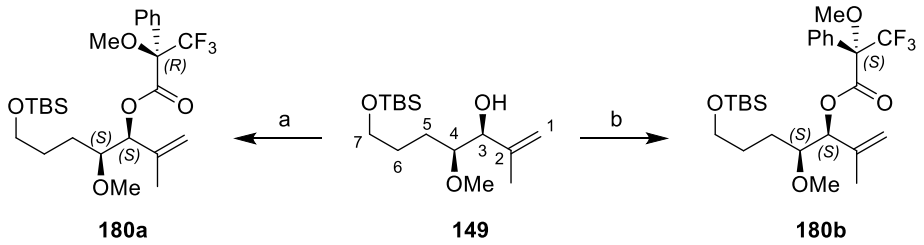
Scheme 32. Synthesis of the eastern fragment **149**. Reaction conditions: a) $NaNO_2$, HCl, H_2O, 0 °C for 2 h, then RT for 12 h, 53%; b) $BH_3 \cdot SMe_2$, THF, RT, 3 h, 97%; c) PMB-trichloroacetimidate (**179**), CSA, CH_2Cl_2, RT, 24 h, 60%; d) $LiAlH_4$, THF, 0 °C, 30 min, 78%; e) TBSCl, imidazole, CH_2Cl_2, RT, 1 h, 90%; f) NaH, MeI, THF, RT, 24 h, 94%; g) DDQ, CH_2Cl_2/H_2O, RT, 1.5 h, 89%; h) DMP, $NaHCO_3$, CH_2Cl_2, RT, 1 h, 99%; i) See Table 5.

Entry	Reaction conditions	d.r. = *syn* : *anti* (yield)
1	Isopropenylmagnesium bromide, $MgBr_2 \cdot Et_2O$, CH_2Cl_2, −78 °C for 3 h, then RT	> 95 : 5 (53%)
2	Isopropenylmagnesium bromide, THF, −78 °C for 3 h, then RT	> 95 : 5 (56%)

Table 5. Optimization of the Grignard reaction.

To determine the absolute configuration of the stereogenic center at C-3, the secondary alcohol was transformed into the corresponding Mosher esters **180a** and **180b** using (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chlorides, respectively (Scheme 33). The obtained Mosher esters were analyzed using ^{1}H NMR spectroscopy according to the Hoye's protocol which bases on the fact that the MTPA esters display different chemical shifts of protons at the R^1 and R^2 substituents.[146] The absolute configuration of the

stereogenic center at C-3 could be determined by analysis of the sign of the difference in chemical shifts for analogous pairs of protons (Table 6).



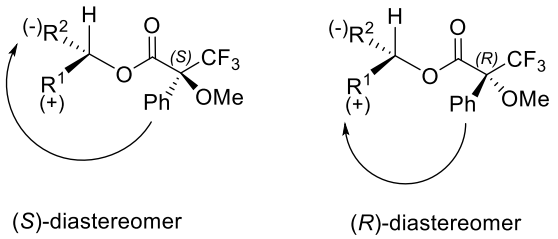
180a **149** **180b**

Scheme 33. Synthesis of the Mosher esters **180a** and **180b**. Reaction conditions: a) (*S*)-(+)-MTPA-Cl, Et_3N, DMAP, CH_2Cl_2, RT, 4 h, 84%; b) (*R*)-(−)-MTPA-Cl, Et_3N, DMAP, CH_2Cl_2, RT, 4 h, 82%.

Number ^{1}H	δ (*S*)-ester [ppm]	δ (*R*)-ester[ppm]	$\Delta\delta^{SR} = \delta S\text{-}\delta R$
2-Me	1.63	1.76	−0.13
H-1a	5.08	5.13	−0.05
H-1b	5.01	5.05	−0.04
H-4	3.42	3.33	+0.09
OMe	3.36	3.16	+0.20

Table 6. Mosher ester analysis for the determination of the absolute configuration of the compound **149** using ^{1}H NMR.

Based on empirical and computationally calculated models of conformations of the MTPA esters it has been known that the phenyl substituent imposes magnetic shielding effect on protons residing on or in close proximity to the axis perpendicular to the aryl ring (Scheme 34).[146]

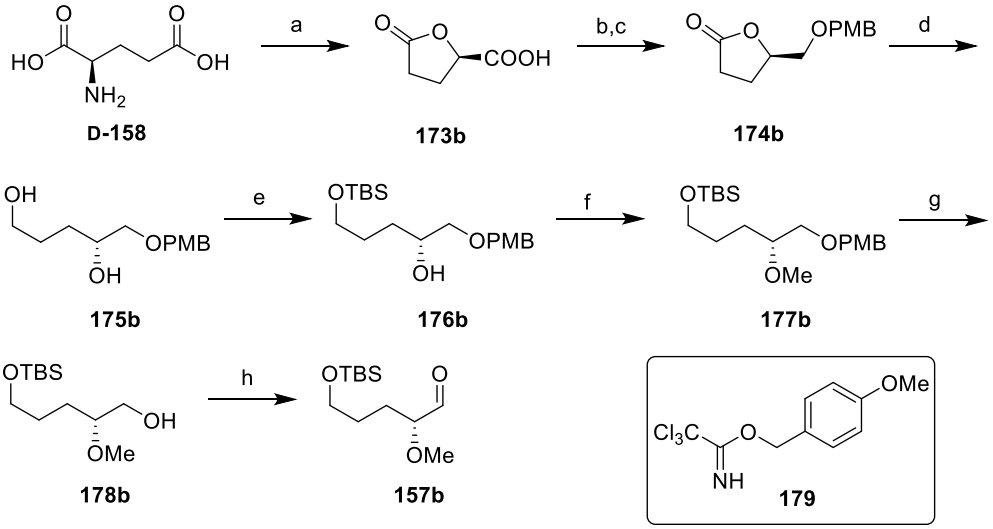


Scheme 34. Conformations used for the analysis of the Mosher esters.

The protons of the R^2 substituent are more shielded in the (*S*)-diastereomer which results in a more upfield chemical shift, while in the (*R*)-diastereomer the protons of the R^1 group are more shielded. As a consequence, the sign of the $\Delta\delta^{SR}$ value for protons of the R^1 is positive and for those in R^2 the sign is negative. In case of the eastern fragment **149** the sign of the $\Delta\delta^{SR}$ value was positive for the proton H-4 and the methoxy group protons which indicates the *S* configuration of the stereogenic center at C-3.

2.3.4.2 Synthesis of the Eastern Fragment 150a

The synthesis of the aldehyde **157b** is analogous to the preparation of the aldehyde **157a**. It started from commercially available D-glutamic acid (**158**-D) which was subjected to diazotization to give the lactone **173b** in 58% yield (Scheme 35).

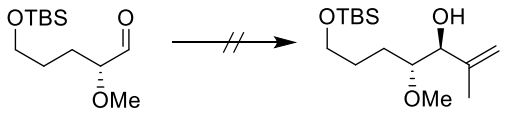


Scheme 35. Synthesis of the aldehyde **157b**. Reaction conditions: a) $NaNO_2$, HCl, H_2O, 0 °C for 2 h, then RT for 12 h, 58%; b) $BH_3 \cdot SMe_2$, THF, RT, 3 h, 98%; c) PMB-trichloroacetimidate (**179**), CSA, CH_2Cl_2, RT, 24 h, 75%; d) $LiAlH_4$, THF, 0 °C, 30 min, 73%; e) TBSCl, imidazole, CH_2Cl_2, RT, 1 h, 92%; f) NaH, MeI, THF, RT, 24 h, 88%; g) DDQ, CH_2Cl_2/H_2O, RT, 1.5 h, 89%; h) DMP, $NaHCO_3$, CH_2Cl_2, RT, 1 h, 99%.

The following reduction with borane-dimethyl sulfide complex and PMB-protection with *p*-methoxybenzyl trichloroacetimidate (**179**) provided the compound **174b** in good yields. The lactone **174b** was then reduced with lithium aluminium hydride in 73% yield to give the diol **175b** which was used in further syntheses of two other eastern fragments **151** and **152**. The primary alcohol in the compound **175b** was protected using *tert*-butyldimethylsilyl chloride in 92% yield. The methylation of the hydroxyl group with methyl iodide proceeded in 88% yield

after which the following PMB-deprotection using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone provided the compound **178b**. Subsequently, the alcohol **178b** was oxidized to the desired aldehyde **157b** in 99% yield using the DMP reagent.[135]

As it was shown for the eastern fragment **149**, the Grignard addition to the aldehyde **157a** underwent predominantly with *syn*-selectivity. In this case, the *anti*-diastereomer **150a** was the desired one. Despite altering the reaction temperature and changing the solvent, the desired diastereoselectivity could not be achieved when isopropenylmagnesium bromide was employed (Table 7). When the aldehyde **157b** was treated with isopropenylmagnesium bromide at −20 °C, the diastereomeric ratio amounted to 1 : 3 *anti* : *syn* (Entry 1). After lowering the temperature to −78 °C the ratio *anti* : *syn* remained unchanged (Entry 2). In another attempt the Grignard reagent was added to the aldehyde **157b** in Et_2O at −90 °C but this only improved undesired *syn*-selectivity (Entry 3).



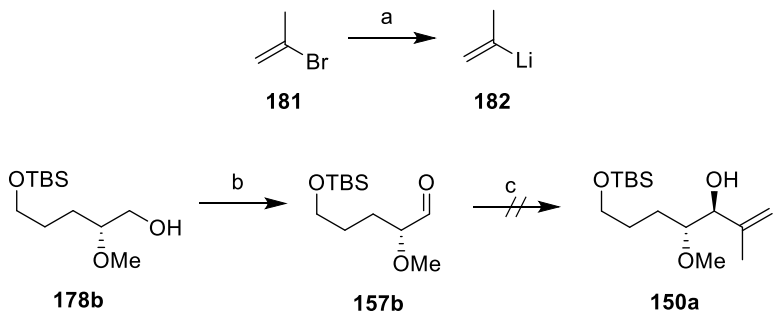
157b **150a**

Entry	Reaction conditions	d.r. (*anti* : *syn*)
1	Isopropenylmagnesium bromide, Et_2O, −20 °C for 2 h, then RT	1 : 3
2	Isopropenylmagnesium bromide, Et_2O, −78 °C for 3 h, then RT	1 : 3
3	Isopropenylmagnesium bromide, Et_2O, −90 °C for 3 h, then RT	1 : 5

Table 7. Optimization of the Grignard reaction.

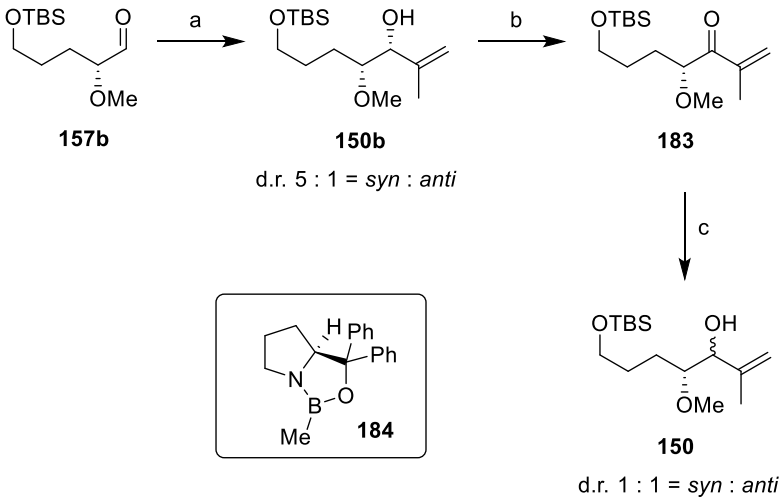
As the modification of the reaction conditions with the use of isopropropenymagnesium bromide did not provide a desired *anti*-selectivity, another approach has been taken into account (Scheme 36).

Isopropenyllithium (**183**) has been prepared from lithium and 2-bromopropene (**181**) according to the procedure described by Leonard.[147] The alcohol **178b** was oxidized to the aldehyde **157b** using the DMP reagent and treated with the prepared isopropenyllithium. Unfortunately, neither the *anti*-diastereomer **150a** nor the *syn*-product **150b** could be detected in the post-reaction mixture.



Scheme 36. Unsuccessful approach towards the eastern fragment **150a**. Reaction conditions: a) 2-bromopropene (**181**), lithium, Et_2O, RT, 3 h; b) DMP, $NaHCO_3$, CH_2Cl_2, RT, 1 h, 98%; c) isopropenyllithium (**182**), Et_2O, −78 °C, 3 h.

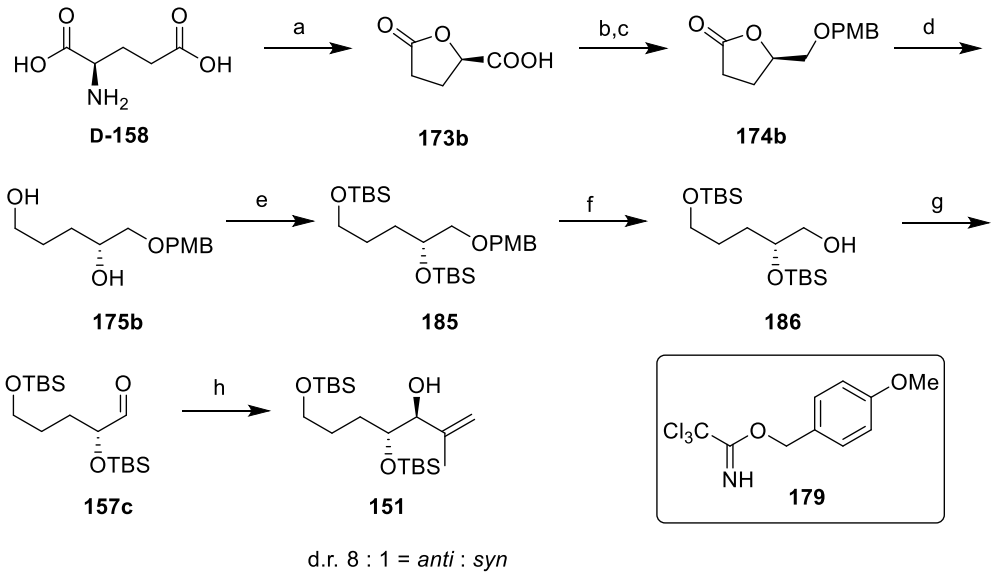
Subsequently, a reduction reaction with chiral oxazoborolidinone catalyst **184** was tested (Scheme 37).[148,149] Isopropenylmagnesium bromide was added to the aldehyde **157b** providing the compound **150b** with diastereomeric ratio *syn* : *anti* = 5 : 1 which was negligible at this point. The alcohol **150b** was then oxidized to the ketone **183** in 86% yield with the use of the DMP reagent. The following reduction of the carbonyl group of **183** using the (*S*)-Corey-Bakshi-Shibata reagent (**184**) provided a 1 : 1 mixture of *syn*- and *anti*-products **150**. The desired *anti*-diastereomer **150a** was purified by flash chromatography and obtained in 22% yield. Finally, the eastern fragment **150a** has been prepared in 2.1% yield over eleven steps.



Scheme 37. Synthesis of the eastern fragment **150a**. Reaction conditions: a) isopropenylmagnesium bromide, Et_2O, −90 °C, RT, 3 h, 50%, d.r. *syn* : *anti* = 5 : 1; b) DMP, $NaHCO_3$, CH_2Cl_2, RT, 1 h, 86%; c) (*S*)-CBS (**184**), $BH_3 \cdot SMe_2$, THF, −78 °C, 24 h, d.r. *syn* : *anti* = 1 : 1; yields after separation: 25% for *syn* product, 22% for *anti* product.

2.3.4.3 Synthesis of the Eastern Fragment 151

The eastern fragment **151** was synthesized from a commercially available D-glutamic acid (**158**-D) (Scheme 38). The synthetic pathway of the diol **175b** was described in the previous section. Both hydroxyl groups in the compound **175b** were protected with the use of *tert*-butyldimethylsilyl trifluoromethanesulfonate affording the compound **185** in 82% yield. The following deprotection of the PMB-group provided the alcohol **186** in 80% yield. The compound **186** was oxidized using the DMP reagent to give the aldehyde **157c** in 99% yield.[135] The subsequent Grignard addition with the use of isopropenylmagnesium bromide provided the desired eastern fragment **151** in 50% yield and with a diastereomeric ratio *anti* : *syn* = 8 : 1. Finally, the compound **151** has been prepared in 13.9% yield over eight steps.



Scheme 38. Synthesis of the eastern fragment **151**. Reaction conditions: a) $NaNO_2$, HCl, H_2O, 0 °C for 2 h, then RT for 12 h, 58%; b) $BH_3 \cdot SMe_2$, THF, RT, 3 h, 98%; c) PMB-trichloroacetimidate (**179**), CSA, CH_2Cl_2, RT, 24 h, 75%; d) $LiAlH_4$, THF, 0 °C, 30 min, 73%; e) TBSOTf, 2,6-lutidine, CH_2Cl_2, RT, 2.5 h, 82%; f) DDQ, CH_2Cl_2/H_2O, RT, 1.5 h, 80%; g) DMP, $NaHCO_3$, CH_2Cl_2, RT, 1 h, 99%; h) isopropenylmagnesium bromide, Et_2O, −90 °C, 35 min, 50%, d.r. *anti* : *syn* = 8 : 1.

The eastern fragment **151** has been predominantly formed as an *anti*-diastereomer (Figure 21), contrary to the synthesis of the eastern fragment **150** in which this selectivity was difficult to achieve. The bulky *tert*-butyldimethylsilyloxy group blocks the Cram-chelate pathway and favors the formation of the Felkin product. Similar observations have been reported by Shen and co-workers in their studies on the chelation-controlled isopropenylation of α-oxygenated

aldehydes.[150] Switching the α-oxygenated substituent from methoxy to a more bulky α-OTBS group resulted in reversed selectivity and formation of the Felkin product.

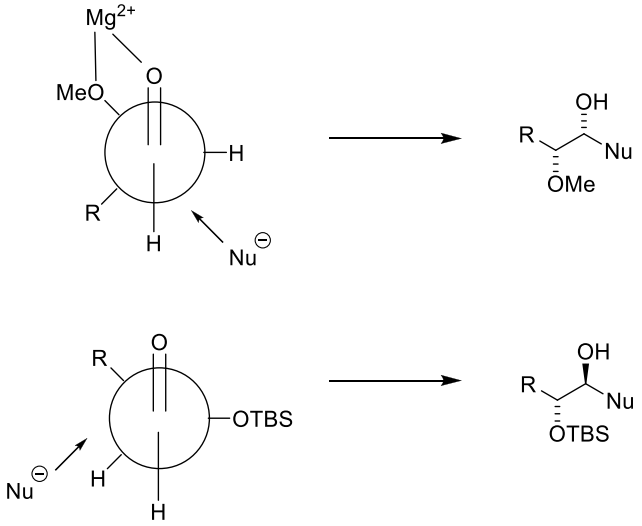
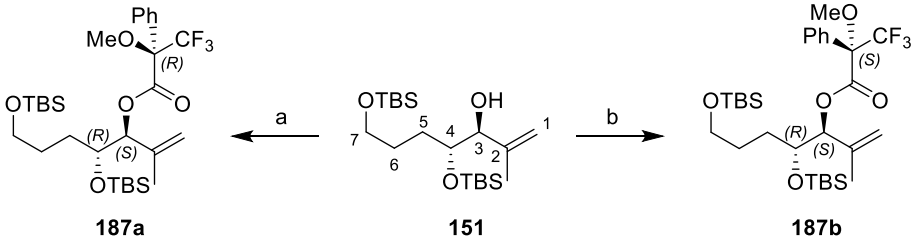


Figure 21. Diastereoselectivity in the Grignard reaction.

To confirm the absolute configuration of the stereogenic center at C-3, the secondary alcohol was transformed into the Mosher esters **187a** and **187b** using (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chlorides, respectively (Scheme 39).



Scheme 39. Synthesis of the Mosher esters **187a** and **187b**. Reaction conditions: a) (*S*)-(+)-MTPA-Cl, Et_3N, DMAP, CH_2Cl_2, RT, 3 h, 79%; b) (*R*)-(−)-MTPA-Cl, Et_3N, DMAP, CH_2Cl_2, RT, 3 h, 70%.

Similarly to the Mosher esters **180a** and **180b**, the absolute configuration at C-3 has been determined by analysis of the sign of the difference in chemical shifts for analogous pairs of protons in the ^{1}H NMR spectra according to the Hoye's protocol (Table 8).[146] In the eastern fragment **151** the sign of $\Delta\delta^{SR}$ value was positive for the protons H-3 and H-4 and negative for H-1a, H-1b and the methyl group 2-Me which indicates the *S* configuration of the stereogenic center at C-3.

Number ^{1}H	δ (*S*)-ester [ppm]	δ (*R*)-ester[ppm]	$\Delta\delta^{SR} = \delta S\text{-}\delta R$
2-Me	1.69	1.78	−0.09
H-1a	4.86	5.02	−0.16
H-1b	4.74	5.00	−0.26
H-4	3.90	3.85	+0.05
H-3	5.45	5.38	+0.07

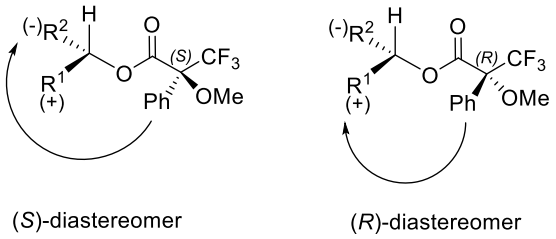
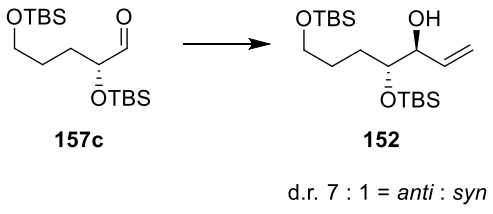


Table 8. Mosher ester analysis for the determination of the absolute configuration of the compound **151** using ^{1}H NMR.

2.3.4.4 Synthesis of the Eastern Fragment 152

The eastern fragment **152** was prepared from the aldehyde **157c** described in the previous section (Scheme 38). The Grignard reaction with the use of vinylmagnesium bromide provided the desired compound **152** in 60% yield and with diastereomeric ratio *anti* : *syn* = 7 : 1 (Scheme 40). The eastern fragment **152** was previously described in the literature by Ōmura and co-workers and the Grignard reaction was optimized by Jekaterina Hermane towards better *anti*-selectivity.[135,151] Finally, the compound **152** has been prepared in 12% yield over eight steps.

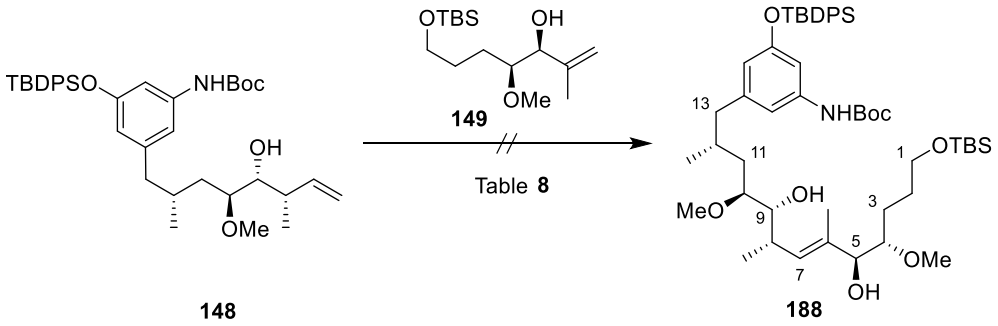


Scheme 40. Synthesis of the eastern fragment **152** from the aldehyde **157c**. Reaction conditions: vinylmagnesium bromide, Et_2O, −90 °C, 35 min, 60%, d.r. *anti* : *syn* = 7 : 1.

2.3.5 Endgame of the Synthesis

2.3.5.1 Olefin Metathesis Reaction

One of the last steps in the synthesis of the desired *seco*-progeldanamycin acid derivatives was olefin cross-metathesis reaction between the western fragment **148** and the corresponding eastern fragments **149-152**. To suppress a possible homodimerization of either of the olefins, one of the fragments ought to be used in excess. As the synthesis of the western fragment **148** has been more time consuming, challenging and expensive, the corresponding eastern fragments were used in excess.



Entry	Catalyst	**149** [eq.]	Solvent	*T* [° C]	Time [h]
1	Grubbs 2nd (**189**)	1.7	CH_2Cl_2	40	48
2	Grubbs 2nd (**189**)	3.0	CH_2Cl_2	40	48
3	Grubbs 2nd (**189**)	3.0	CH_2Cl_2 (degassed)	40	24
4	Grubbs 2nd (**189**)	3.0	Toluene (degassed)	80	24
5	Hoveyda-Grubbs 2nd (**190**)	1.7	CH_2Cl_2	40	48
6	Hoveyda-Grubbs 2nd (**190**)	3.0	CH_2Cl_2	40	48
7	Stewart-Grubbs (**191**)	3.0	CH_2Cl_2	40	48
8	Catalyst **192**	3.0	CH_2Cl_2	40	48
9	Nitro-Grela (**193**)	3.0	CH_2Cl_2	40	48
10	Nitro-Grela (**193**)	3.0	Toluene	80	48
11	CAZ-1 (**194**)	3.0	CH_2Cl_2	40	48
12	CAZ-1 (**194**)	3.0	Toluene	80	48

Table 9. Screening of different catalysts for the olefin metathesis reaction. In each case the second portion of the catalyst was added after 10-12 h.

The approach towards the construction of the trisubstituted olefin in the geldanamycin (**4**) synthesis has been already reported by Andrus and co-workers.[152] However, a challenging ring-closing metathesis involving a combination of a large ring and a trisubstituted olefin target was unsuccessful. The first metathesis reaction towards the compound **188** was tested between the western fragment **148** and the eastern fragment **149** (Table 9). A variety of conditions, including different catalysts (Figure 22), solvents, temperature and a number of eastern fragment equivalents was screened.

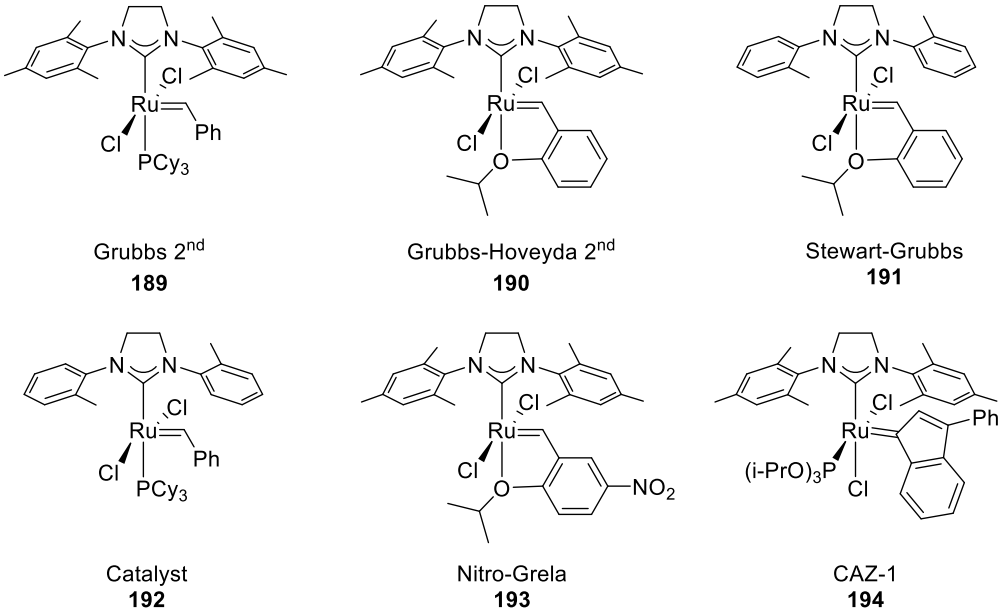
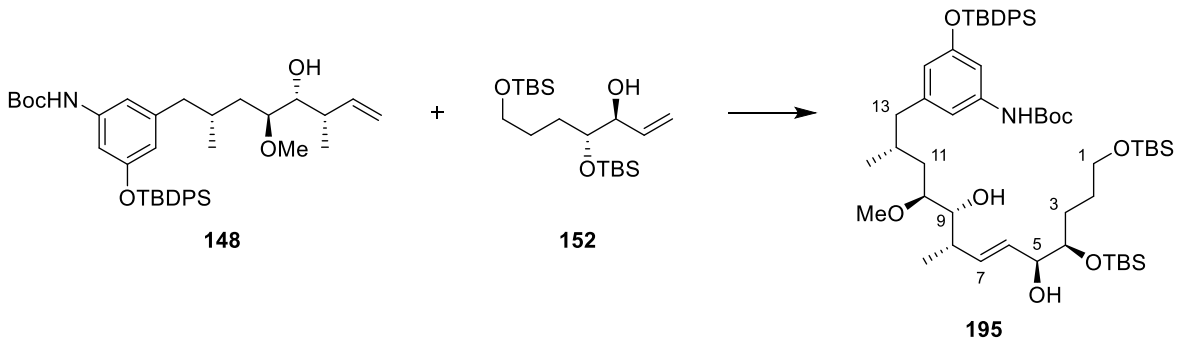


Figure 22. Structures of catalysts tested in the metathesis reactions.

The Grubbs[2nd] catalyst (**189**) was explored as the first catalyst in four reactions carried out in CH_2Cl_2 or toluene with addition of 1.7 or 3 equivalents of the eastern fragment **149** (Entries 1-4). Unfortunately, none of these reactions was successful and did not lead to the desired compound **188**. The next catalyst used in the catalyst screening was the Grubbs-Hoveyda[2nd] catalyst (**190**) which was proven to be successful in the synthesis of the compounds **144**-**146** prepared by Jekaterina Hermane.[135] Unfortunately, also in this case no desired product **188** was formed, probably due to the presence of an additional methyl group at C-6 when compared to the structures **144**-**146** (Entries 5-6). In the next two attempts, the Stewart-Grubbs catalyst (**191**) and the catalyst **192** were used and the reactions were carried out in CH_2Cl_2 (Entries 7-8). Both catalysts have exhibited high efficiency in the preparation of tetrasubstituted olefins by cross-metathesis of sterically hindered olefins,[153] however, the synthesis of the trisubstituted olefin **188** was unsuccessful. The nitro-analogue of Hoveyda-

Grubbs^{2nd} catalyst (**193**)[154], improved by changing the electronic properties of the Ru-chelating fragment was used in the next two reactions (Entries 9-10). As with the catalyst CAZ-1 (**194**; Entries 11-12) and most of the above-described reactions, the substrates could be recovered from the post-reaction mixture while the formation of the trisubstituted olefin **188** possessing a TBS substituent was not observed.

Since the eastern fragments **150**-**151** also possess the methyl group at C-2, the metathesis reaction between the western fragment **148** and the corresponding olefins **150**-**151** has not been further explored. To synthesize the SNAc ester of 8-*des*-methyl-*seco*-progeldanamycin **137**, a cross-metathesis reaction between the western fragment **148** and the eastern fragment **152** was carried out (Scheme 41). This reaction was optimized by Jekaterina Hermane and employed in the synthesis of the SNAc ester **146**.[135] The olefin **148** reacted with the alkene **152** in the presence of the Grubbs-Hoveyda 2nd catalyst (**190**). The reaction was performed in CH_2Cl_2 at 40 °C for 48 h and provided the desired product **195** in 40% yield. The coupling constant between H-6 and H-7 protons which was determined to be J = 15.2 Hz indicated an *E*-double bond in the prepared product.

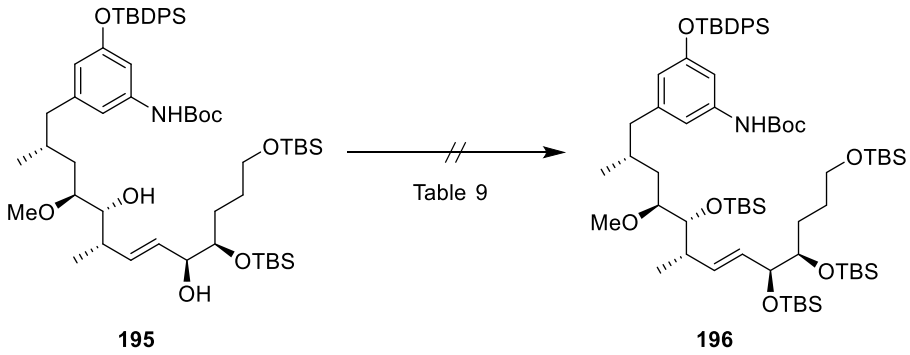


Scheme 41. Synthesis of **195**. Conditions: Grubbs-Hoveyda 2nd (**190**), CH_2Cl_2, 40 °C, 48 h, 40%.

2.3.5.2 Synthesis of the SNAc ester of 8-*des*-methyl-*seco*-progeldanamycin 137

It has been known from the previous work of Jekaterina Hermane that open chain *seco*-acid derivatives and thioesters are unstable, while removal of the protecting groups may cause undesired reactions or degradation. Therefore, for the last steps of the total synthesis the mildest possible conditions had to be employed. All the reactions were controlled by mass spectrometry and TLC to monitor the progress of the reaction and when it had to be terminated.

First, the hydroxyl groups in the metathesis product **195** were planned to be protected with *tert*-butyldimethylsilyl trifluoromethanesulfonate (Table 10). Unfortunately, the results of these reactions at different conditions were not fruitful and did not provide the desired product **196**. After the first attempt with the use of 5 equivalents of 2,6-lutidine and 3 equivalents of TBSOTf, a single TBS-protection was observed (Entry 1). When the number of equivalents was increased to 6.5 and 4 for 2,6-lutidine and TBSOTf, respectively, both hydroxyl groups were protected but a simultaneous deprotection of the Boc group took place (Entry 2). A longer reaction with lower amounts of the reagents gave the same result (Entry 3). Unfortunately, increasing the number of equivalents along with a longer reaction time only resulted in decomposition of the substrate (Entry 4).

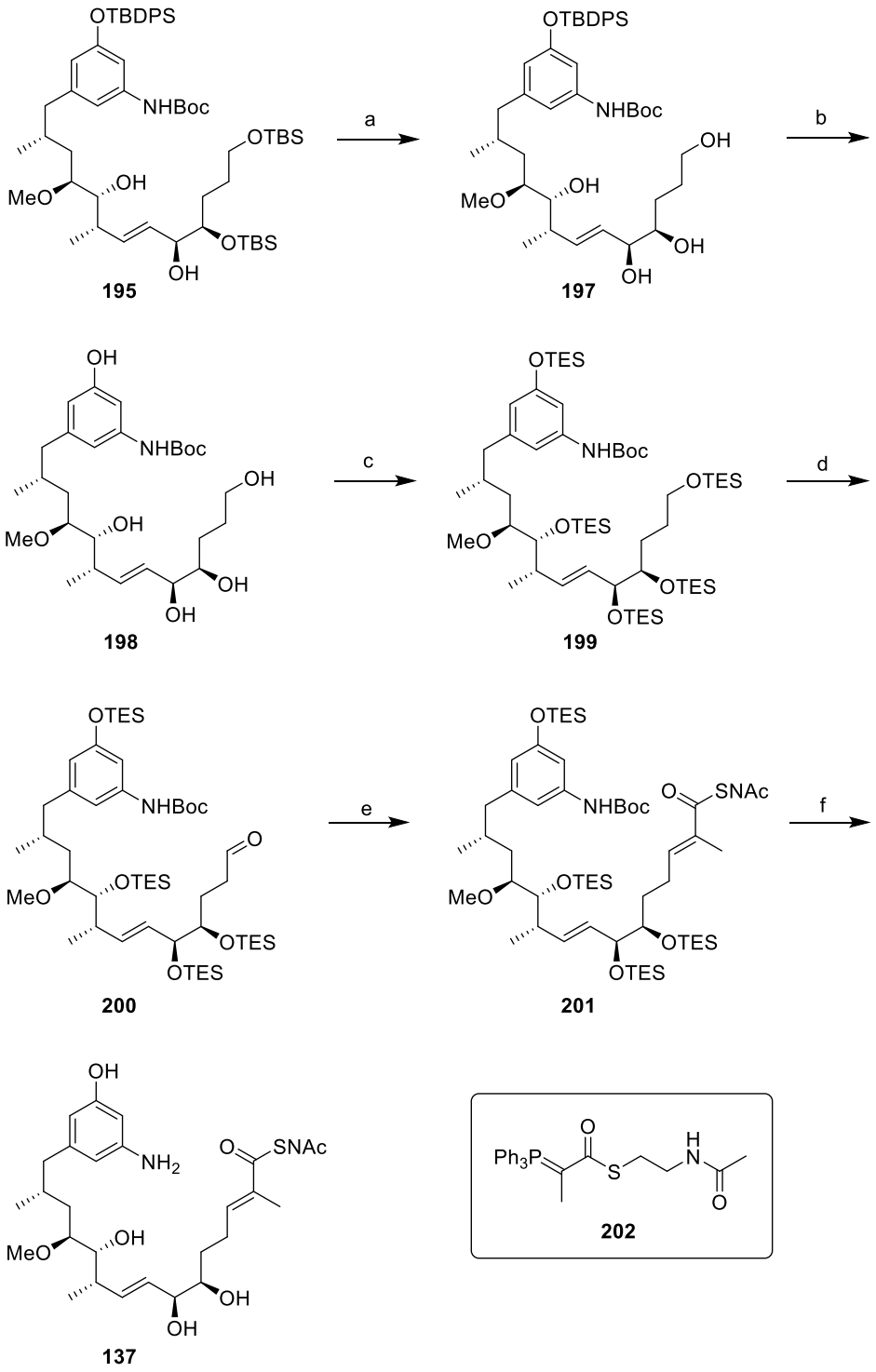


195 **196**

Entry	Reaction conditions	Result
1	RT, 3 h, CH_2Cl_2, TBSOTf (3.0 eq.), 2.6-lutidine (5.0 eq.)	Single TBS-protection (71%)
2	RT, 3 h, CH_2Cl_2, TBSOTf (4.0 eq.), 2.6-lutidine (6.5 eq.)	Boc-deprotection (26%)
3	RT, 12 h, CH_2Cl_2, TBSOTf (3.0 eq.), 2.6-lutidine (5.0 eq.)	Boc-deprotection (26%)
4	RT, 12 h, CH_2Cl_2, TBSOTf (3.5 eq.), 2.6-lutidine (6.0 eq.)	decomposition

Table 10. Unsuccessful TBS-protection reactions.

Due to the problems with the TBS-protection reactions, another approach towards 8-*des*-methyl-*seco*-progeldanamycin **137** was implemented (Scheme 42). The TBS groups were removed using pyridinium *p*-toluenesulfonate to give the polyol **197** in 90% yield. The following TBDPS-deprotection was carried out using tetra-*n*-butylammonium fluoride and optimized as presented in Table 11. The first two attempts with the use of 3 equivalents of $TBAF \cdot 3H_2O$ at 0 °C and RT did not provide the desired product **198** whereas the substrate **197** could be recovered from the post-reaction mixture (Entries 1-2).



Scheme 42. Synthesis of *seco*-progeldanamycin derivative **137**. Reaction conditions: a) PPTS, MeOH, 40 °C, 48 h, 90%; b) See Table 11; c) TESOTf, 2,6-lutidine, CH_2Cl_2, −78 °C → RT, 1 h, 82%; d) $(COCl)_2$, DMSO, DIPEA, CH_2Cl_2, −78 °C, 1.5 h, 80%; e) **202**, $CHCl_3$, 40 °C, 48 h, 86%, E/Z > 10 : 1; f) TFA, 0 °C, 3 h, 73%.

Also no product was observed for the next two reactions: with the use of a 1 M solution of TBAF in THF at −78 °C and at RT. However, in these cases the polyol **197** decomposed. The

use of 1.5 or 3 equivalents of TBAF·3H$_2$O provided only traces of the product **198** (Entries 5-6). Finally, the TBDPS-deprotected product **198** was obtained in 80% yield in the reaction carried out using 5 equivalents of TBAF·3H$_2$O at −78 °C for 20 h (Entry 7).

Entry	Reaction conditions	Result
1	0 °C, 5 h, THF, TBAF·3H$_2$O (3.0 eq.)	No product, substrate recovered
2	RT, 5 h, THF, TBAF·3H$_2$O (3.0 eq.)	No product, substrate recovered
3	−78 °C, 20 h, THF, TBAF (1 M in THF, 5.0 eq.)	No product, substrate decomposed
4	RT, 20 h, THF, TBAF (1 M in THF, 5.0 eq.)	No product, substrate decomposed
5	−78 °C, 3 h, THF, TBAF·3H$_2$O (1.5 eq.)	Traces of the product
6	−78 °C, 3 h, THF, TBAF·3H$_2$O (3.0 eq.)	Traces of the product
7	−78 °C, 20 h, THF, TBAF·3H$_2$O (5.0 eq.)	80%

Table 11. Optimization of the TBDPS-deprotection reaction.

All the hydroxyl groups in the polyol **198** were protected using triethylsilyl trifluoromethanesulfonate in 82% yield. The following Swern oxidation reaction provided the aldehyde **200** in 80% yield which was converted to the SNAc ester **201** using phosphonium ylide **202** in 86% yield. The ylide **202** was prepared according to the Hahn's procedure in a two-step synthesis starting from 2-bromopropanoic acid.[155] The final deprotection of the Boc- and TES-groups with the use of trifluoroacetic acid provided the desired 8-*des*-methyl-*seco*-progeldanamycin acid derivative **137** in 73% yield. Overall, the compound **137** has been prepared in twenty-six steps (longest linear sequence) in the total yield of 5.6%.

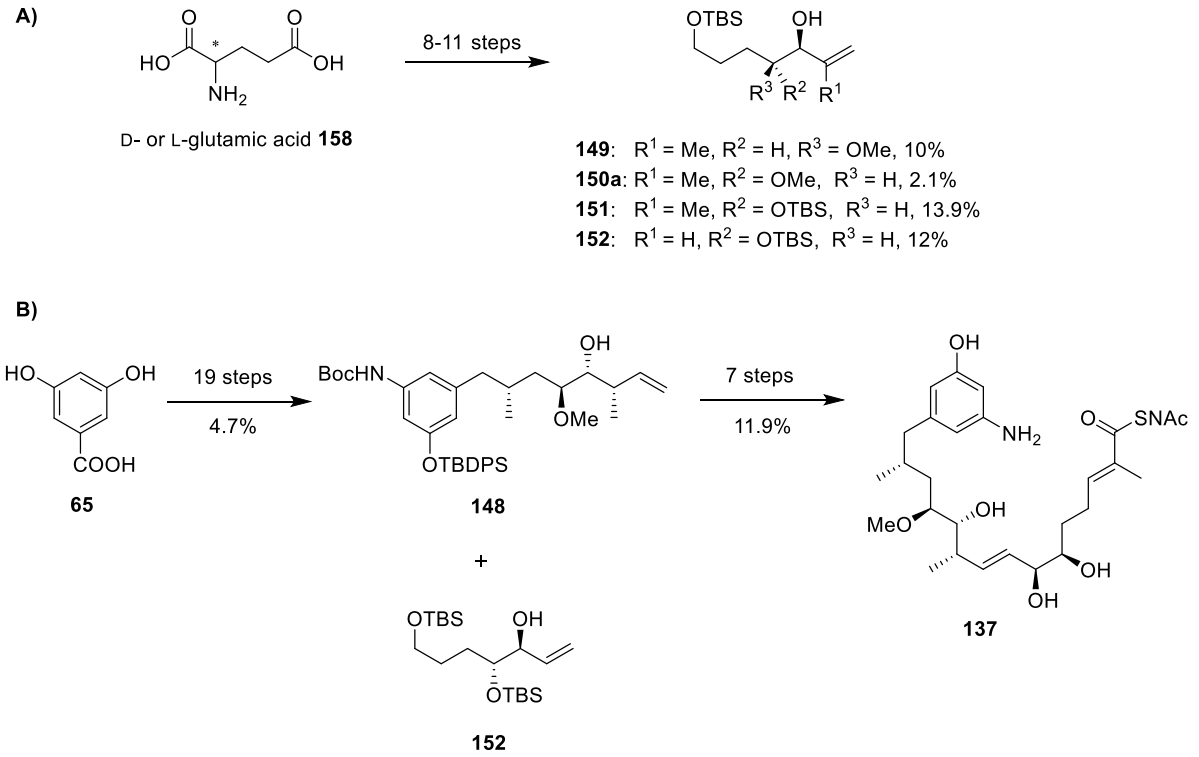
2.4 Summary and Outlook

2.4.1 Summary

The second part of the doctoral thesis was focused on another ansamycin antibiotic – geldanamycin. The total synthesis of new *seco*-progeldanamycin acid derivative was carried out to provide a substrate for the studies on the flexibility of the amide synthase (GdmF), a key enzyme in the geldanamycin biosynthesis responsible for the macrolactamization.

The desired progeldanamycin derivative has been synthesized in twenty-six steps (longest linear sequence) in the overall yield of 5.6% (Scheme 42). On the basis of the previous work, the western fragment was prepared from 3,5-dihydroxybenzoic acid in nineteen steps in the

total yield of 4.7%. The key steps in the synthesis of this fragment were Sharpless epoxidation, Evans alkylation and Roush crotylation for the construction of the stereocenters at C-10 and C-11.



Scheme 43. A) Synthesis of the eastern fragments **149**-**152**. B) Synthesis of 8-*des*-methyl-*seco*-progeldanamycin acid derivative **137**.

The eastern fragments were synthesized from D- of L-glutamic acid in 2-10% overall yields. The terminal double bond was installed by either *syn*- or *anti*-selective Grignard addition. To connect the western and eastern fragments olefin cross-metathesis reaction was carried out, while the introduction of the SNAc ester was achieved by the Wittig reaction.

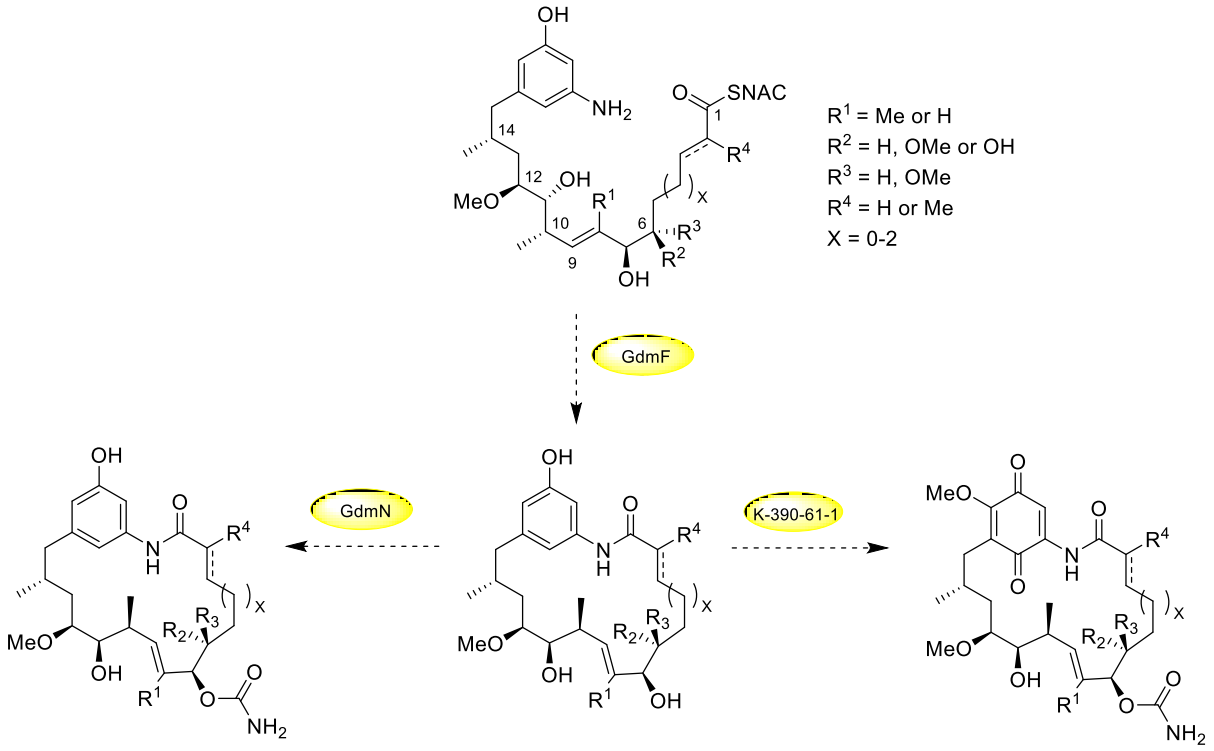
The *seco*-progeldanamycin acid derivative **137** has been obtained as one of the four compounds which had been planned to be synthesized. The most probable reason of the unsuccessful metathesis reaction was an additional methyl group at the terminal double bond of the eastern fragment which renders the sought trisubstituted olefin a much more difficult target to be achieved.

The 8-*des*-methyl-*seco*-progeldanamycin acid derivative **137** is planned to be used in enzyme expression studies with the heterologously expressed amide synthase (GdmF) to investigate the tolerance and biosynthetic transformation by the enzyme. If the obtained amount of the

compound were larger, the prepared SNAc ester could be fed to a knockout strain of *Streptomyces hygroscopicus var. geldanus* (K-390-61-1) to obtain new geldanamycin derivative.

2.4.2 Outlook of the Project

For the future studies on the flexibility of the amide synthase (GdmF) more substrates should be prepared in order to investigate the tolerance and biosynthetic transformation by the enzyme (Scheme 44).



Scheme 44. Possible pathways for the further investigation towards the substrate specificity of the amide synthase GdmF.

The substrates could be modified at the positions C-2, C-6 and C-8. It has been known that the substituents at C-6 may affect toxicity, while modifications at C-8 should not have a significant impact on the anticancer activity of geldanamycin derivatives. The introduction of the methyl group at C-8 requires further optimization (e.g. relay metathesis) or new synthetic routes as all the attempts with the use of cross-metathesis reaction, unfortunately, failed. Additionally, an impact of alterations at C-10 and C-12 positions could be investigated. On the other hand, the synthesis could also be focused on the substrates which enable the production of 17- to 20-membered macrolactams.

The prepared progeldanamycin derivatives could be fed to the blocked mutant of *S. hygroscopicus* (K390-61-1) to obtain new geldanamycin derivatives. Additionally, the carbamoyl transferase (GdmN) could be expressed to investigate the carbamoylation of progeldanamycin derivatives. Following tests of biological activity of these compounds could provide essential information for the structure-activity relationship.

3 Experimental Part

3.1 Materials and Methods

Reagents and Solvents

Commercially available reagents (Acros Organics, Sigma Aldrich, ABCR, TCI) and solvents were used as received or purified by conventional methods prior to use as described in the literature.[156]

Thin Layer and Column Chromatography

Precoated silica gel 60 F254 plates (Merck, Darmstadt) were used for TLC and the spots were visualized with UV light at 254 nm or alternatively by staining with ninhydrine or potassium permanganate. Flash column chromatography was performed using silica gel MACHEREY-NAGEL (grain size 40-63 μm).

NMR Spectroscopy

^{1}H NMR spectra were recorded with a Bruker 200 MHz spectrometer with DPX console, Bruker Avance-400 (400 MHz) with DPC console and Bruker DRX-500 (500 MHz) with DRX-console at room temperature. ^{13}C NMR spectra were recorded at 100 MHz with a Bruker Avance-400 and at 125 MHz with a Bruker DRX-500. Chemical shifts are reported in δ (ppm) relative to TMS as the internal standard. Multiplicities are described using the following abbreviations: s – singlet, d – doublet, t – triplet, q – quartet, m – multiplet, b – broad. In ^{13}C NMR following descriptors have been used for the order of the carbon atom: s – quaternary, d – tertiary, t – secondary, q – primary. All coupling constants *J* are expressed in Hertz.

HPLC

High-performance liquid chromatography was performed using a VARIAN PROSTAR system (pump Prepstar Model 218, variable wavelength detector Prostar; preferred monitoring at λ = 248 nm) with the column TRENTEC Reprosil-Pur 120 C18 AQ (5 μm, 250 mm x 25 mm, with guard column 40 mm x 8 mm abbreviated C18-1) with parallel mass spectrometric detection (MICROMASS type ZMD ESI-Quad spectrometer).

Semi-preparative high performance liquid chromatography was performed using a MERCK HITACHI Model 7000 system [pump L-7100, diode array detector L-7450 (λ = 220-400 nm, preferred monitoring at λ = 248 nm)] with the column NUCLEODUR C18 ISIS (5 μm, 250 mm

x 8 mm; abbreviated C18-2). Operating conditions and retention times (t_R) are reported in the section 5.3.

Mass Spectrometry

Mass spectra were recorded with a type LCT (ESI) equipped with a lockspray dual ion source in combination with a WATERS Alliance 2695 LC system, or with a type QTOF premier (MICROMASS) spectrometer (ESI mode) in combination with a Waters Acquity S2 UPLC system equipped with a Waters Acquity UPLC BEH C18 1.7 μm (SN 01473711315545) column [solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 0.4 mL/min; gradient (*t* [min]/solvent B[%]: 0/5, 2.5/95, 6.5/95, 6.6/5, 8/5)]. Retention times (t_R) are given in the section 3.3. Ion mass signals (*m*/*z*) are reported as values in atomic mass units.

Melting Points

Melting points (M.p.) were determined on a SRS OptiMelt apparatus and are reported uncorrected.

Optical Rotation

Specific optical rotation values $[\alpha]^t_D$ were measured on a polarimeter 341 (PERKIN ELMER) at a wavelength of 589 nm (D) and given temperature *t*.

3.2 Mutasynthetic Experiments

3.2.1 Bacterial Strain

For a mutasynthetic approach a blocked mutant of *Amycolatopsis mediterranei* HGF003 was used. It is a knock-out of *rifK* – a gene encoding AHBA synthase which is indispensable in the biosynthesis of AHBA. Consequently, the bacterial strain is unable to produce rifamycin unless AHBA is added to the culture medium.

3.2.2 Materials and Media

All the work with bacteria was performed using sterile devices and media. The cultivation of *A. mediterranei* HGF003 strain was carried out on agar plates at 28 °C in a Heraeus or a Thermo Scientific Heratherm IGS100 incubator, while the cultivation in liquid medium was performed using a New Brunswick Scientific Innova® 4900 gyratory multi-shaker or a New Brunswick Scientific Innova® 44/44R stackable shaker at 200 rpm at 28 °C. The cultivation media were prepared using distilled water and sterilized by autoclaving: YMG-agar − 10 g/L

malt extract (Sigma Aldrich, Roth), 4 g/L yeast extract (Bacto, Duchefa Biochemie), 4 g/L D-(+)-glucose·H_2O (Roth), 22 g/L agar (Bacto, Roth); vegetative medium − 5 g/L beef extract powder (Sigma Aldrich), 5 g/L peptone (Roth), 5 g/L yeast extract (Bacto, Duchefa Biochemie), 2.5 g/L caseine hydrolysate (enzymatic, Roth), 1.5 g/L NaCl (Honeywell), 22 g/L glucose·H_2O; production medium: 25 g/L peanut meal, 9.6 g/L $(NH_4)_2SO_4$, 9.5 g/L $CaCO_3$, 126.5 g/L glucose·H_2O, 1mL/L trace elements solution (1 g/L $MgSO_4 \cdot H_2O$, 1g/L $CuSO_4 \cdot 5H_2O$, 1g/L $FeSO_4 \cdot 7H_2O$, 1g/L $MnSO_4 \cdot H_2O$, 1g/L $Co(NO_3)_2 \cdot 6H_2O$).

3.2.3 Cultivation Parameters

Amycolatopsis mediterranei HGF003 was stored as spore suspensions in a 40% *v*/*v* glycerol/water solution at –80 °C and used for the inoculation of agar plates. For novel mutasynthons test fermentations were first carried out on 1-5 agar plates. When the acceptance of the substrate was confirmed by UPLC-MS, large-scale fermentation on 40 agar plates or in vegetative/production medium was conducted. Simultaneously, the microbial strain was fed with the natural starter building block 3-amino-5-hydroxybenzoic acid (AHBA, **7**) to monitor the productivity of the strain (positive control). The inoculated agar plates or flask charged with vegetative medium with no mutasynthon added constituted the zero control.

Test fermentations were carried out on agar plates at 28 °C for 14 days. After 7 days 10 mg of a respective substrate dissolved in 1.5 mL of DMSO/water mixture per plate was added by sterile filtration. When cultivation was completed after additional seven days, homogenization by breaking agar plates and extraction with ethyl acetate was carried out. The combined extracts were dried over $MgSO_4$ and the solvent was removed *in vacuo*.

For cultivation in liquid medium, a few colonies were transferred directly from the agar plate after 7 days of growing to 500 mL baffled-bottom flasks or flasks with steel springs containing vegetative medium (50 mL per flask). Precultures were shaken for 3 days at 28 °C before inoculation of the main cultures (500 mL baffled-bottom flask or flask with steel springs charged with 50 mL of production medium, 0.0625 mmol of substrate dissolved in 4 mL of a DMSO/water mixture, preferably 1 : 1). Shaking was continued at 28 °C for 4 days for total cultivation time of 13 days. Scale-up fermentations were carried out in vegetative (50 mL per flask) and production media (1 L, 1.25 mmol/L of substrate in 4 mL of a DMSO/water mixture, 4 mL of inoculum from vegetative medium per flask) as described above.

3.2.4 HPLC and MS Analyses

The purification and fractionation of the fermentation material in the amounts of >10 mg was carried out using preparative high-performance liquid chromatography equipped with a VARIAN PROSTAR system. For the amounts ≤10 mg semi-preparative high performance liquid chromatography with a MERCK HITACHI Model 7000 system was used (section 5.1).

Before HPLC analysis, the crude material was filtered through a column chromatography (silica gel, elution with ethyl acetate) in order to remove the bacteria cells followed by a sequence of chromatographic purifications. Before each injection the sample was dissolved in a 1 : 1 mixture of methanol and water (HPLC grades) and filtered through a PTFE syringe filter Rotilabo (pore size: 0.45 μm, nominal diameter: 13 mm).

For the detection of the products from the fermentation cultures samples from the culture broth (200 μL) were dissolved in methanol (200 μL), centrifuged (1 min, 4 °C) and screened by UPLC-MS (section 5.1).

3.2.5 Preparation of the Spore Suspensions

A. mediterranei strain HGF003 was stored as spore suspensions in a 40% (*v*/*v*) glycerol/water solution at –80 °C. Three weeks after inoculation of the YMG agar plates, 3-4 mL of water was added onto agar plate. The surface of the agar plate was rinsed and the liquid was filtered through a sterile cotton to a sterile falcon. After the subsequent addition of 1-2 mL of water and rinsing the plate, the solution was vortex mixed for ca 45 seconds, filtered through a sterile cotton to a new falcon and centrifuged (4 °C, 5 minutes, 5000 rpm). The supernatant was removed and the obtained precipitation was dissolved in a 40% (*v*/*v*) glycerol/water solution and transferred to a sterile Eppendorf vial. To monitor the productivity of the prepared spores, the microbial strain was fed with the natural starter building block AHBA (3-amino-5-hydroxybenzoic acid) and test fermentations were carried out. For the detection of the expected product – rifamycin B, the crude material (200 μL) was dissolved in methanol (200 μL), centrifuged (1 min, 4 °C) and screened by UPLC-MS. When the productivity of the strain was confirmed by UPLC-MS, the spore suspensions were used for inoculation of YMG agar plates.

3.2.6 Optimization of Medium Components Under Liquid State Fermentation Conditions

For the liquid state fermentation the medium components had to be optimized. For this purpose three sets of media were tested:

1. Vegetative medium: 5 g/L beef extract powder, 5 g/L peptone, 5 g/L yeast extract, 2.5 g/L caseine hydrolysate (enzymatic), 1.5 g/L NaCl, 22 g/L glucose·H_2O.

   Production medium: 25 g/L peanut meal, 9.6 g/L $(NH_4)_2SO_4$, 9.5 g/L $CaCO_3$, 126.5 g/L glucose·H_2O, 1mL/L trace elements solution (1 g/L $MgSO_4·H_2O$, 1g/L $CuSO_4·5H_2O$, 1g/L $FeSO_4·7H_2O$, 1g/L $MnSO_4·H_2O$, 1g/L $Co(NO_3)_2·6H_2O$).

2. Vegetative medium: 20 g/L glucose·H_2O, 3 g/L KH_2PO_4, 1.5 g/L K_2HPO_4, 0.016 g/L $MgSO_4·H_2O$, 0.001 g/L $Zn(OAc)_2$, 5 g/L yeast extract.

   Production medium: 40 g/L glucose·H_2O, 3g/L KH_2PO_4, 1.5 g/L K_2HPO_4, 0.016 g/L $MgSO_4·H_2O$, 0.001g/L $Zn(OAc)_2$, 5g/L yeast extract.

3. Vegetative medium: 20 g/L starch, 15 g/L glucose·H_2O, 10 g/L soybean meal, 10 g/L peptone, 5 g/L KNO_3, 0.3 g/L KH_2PO_4, 3 g/L $CaCO_3$.

   Production medium: 120 g/L glucose·H_2O, 20 g/L soybean meal, 10 g/L peptone, 5 g/L fish meal, 8 g/L KNO_3, 0.5 g/L KH_2PO_4, 1 mg/L $CoCl_2·6H_2O$, 5 g/L $CaCO_3$.

The concentration of rifamycins in the fermentation broth was determined spectrophotometrically according to the Pasqualucci's method.[157] The measurement is possible because of the presence of the naphthoquinone-naphthohydroquinone system which has maximum absorption peak in the visible light region. The general procedure can be summarized as follows: the samples were prepared by taking two 1 mL portions from each fermentation flask. One aliquot was diluted 1 : 6 with buffer A (acetate buffer of pH 4.63 prepared by mixing of 100 mL 1 M NaOH and 200 mL 1 M acetic acid, completing with distilled water to the final volume to 1 L) and the other similarly with buffer B (buffer A containing 0.1% w/v sodium nitrite) as blank. After shaking for 5 minutes, the absorbance of the solution diluted with buffer A was determined at 425 nm in a 1 cm cell against the solution diluted with solution B. The rifamycin B concentration was calculated from the measured absorbance using the formula:

$$\text{rif B}\left(\frac{\mu g}{mL}\right) = \frac{A_{425} \times 50\,000}{21.5}$$

where A_{425} is the absorbance at 425 nm and 21.5 is the absorption of rifamycin B determined by this method.

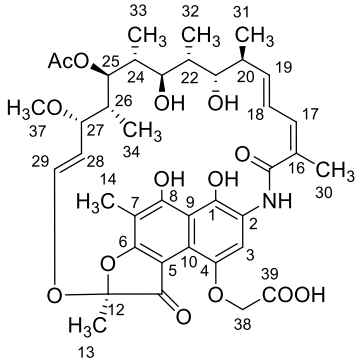
The rifamycin B concentration after the fermentation carried out under conditions described above in the point 1 was calculated as 307 mg/L (A_{425} = 0.132). Under the conditions reported in the point 2 bacteria did not grow. Under fermentation conditions 3 a growth was observed but no rifamycin B was produced.

For additional detection of rifamycin B/novel products, 200 µL of samples from the fermentation broth were mixed with 200 µL of methanol and centrifuged at 14 000 rpm for 5 minutes. The clear supernatant (200 µL) was subjected to UPLC-MS analyses (rifamycin B: HRMS-ESI (*m*/*z*): $[M+H]^+$ calcd for $[C_{39}H_{50}NO_{14}]^+$: 756.3231, found: 756.3228; $[M-H]^-$ calcd for $[C_{39}H_{48}NO_{14}]^-$: 754.3080, found: 754.3057; t_R = 3.2 min; conditions: solvent A = water + 0.1% (v/v) formic acid, solvent B = MeOH + 0.1% (v/v) formic acid; flow rate = 0.4 mL/min; gradient (*t* [min]/solvent B [%]): 0/5; 2.5/95; 6.5/95; 6.6/5; 8/5).

For isolation of novel products from large-scale fermentations, the fermentation broth was extracted three times with ethyl acetate, dried over $MgSO_4$, concentrated under reduced pressure and filtered through silica gel eluting with ethyl acetate followed by removal of the solvent *in vacuo*. The crude fermentation extract was subjected to chromatographic purification steps.

3.3 Characteristic of Rifamycins and the Mutasynthetically Produced Tetraketides

Rifamycin B (1)



Rifamycin B (**1**) was obtained as an orange solid after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-hydroxy-5-nitrobenzoic acid (**95**) (fed 229 mg – 1.25 mmol/L; HRMS, LC-MS/MS), 3-azido-5-hydroxybenzoic acid (**94**) (fed 168 mg – 0.94 mmol/750 mL; isolated amount = 1.5 mg; 2 mg/L) and 3-amino-5-hydroxybenzoic acid (**7**) (fed 191 mg – 1.25 mmol/L; isolated amount = 20 mg/L).

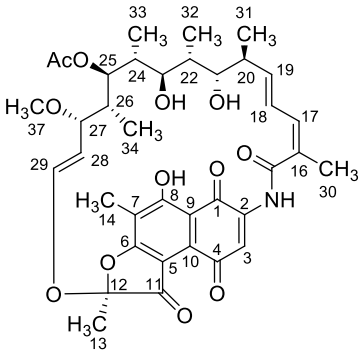
1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 82.0 min.

2. HPLC: preparative HPLC (C18-1): solvent A: water, solvent B: MeOH; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/40; 5/40; 20/50; 50/50; 100/100; t_R = 75.0 min.

3. HPLC: preparative HPLC (C18-1): solvent A: water, solvent B: MeOH; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 85/100; 100/100; t_R = 69.0 min.

UPLC-MS (MeOH) t_R = 3.2 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{39}H_{50}NO_{14}]^+$: 756.3231, found: 756.3228; $[M-H]^-$ calcd for $[C_{39}H_{48}NO_{14}]^-$: 754.3080, found: 754.3057.

Rifamycin S (9)



Rifamycin S (**9**) was obtained as a yellow solid after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-hydroxy-5-nitrobenzoic acid (**95**) (fed 229 mg – 1.25 mmol/L; UPLC-HRMS), 3-azido-5-hydroxybenzoic acid (**94**) (fed 168 mg – 0.94 mmol/750 mL; isolated amount = 1.0 mg; 1.3 mg/L) and 3-amino-5-hydroxybenzoic acid (**7**) (fed 191 mg – 1.25 mmol/L; isolated amount = 1.1 mg/L). Additionally, fermentation with AHBA (**7**) on agar plates (fed 50 mg – 0.33 mmol/125 mL; 10 mg/plate) yielded 0.3 mg of rifamycin S (**9**) (2.4 mg/L).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 77.0 min.

2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 70/55; 90/100; 100/100; t_R = 84.0 min.

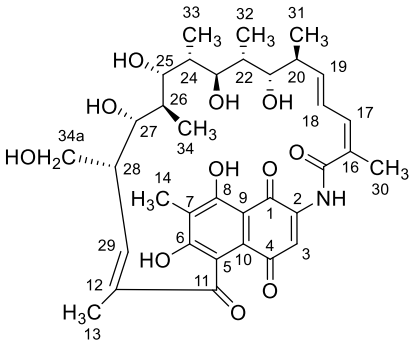
3. HPLC: semi-preparative HPLC (C18-2) solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/60; 100/100; t_R = 68.0 min.

The analytical data are in accordance with the reported in the literature.[158,159]

^{1}H NMR (500 MHz, C_6D_6, C_6D_5H = 7.16 ppm) δ (ppm): 12.73 (s, 1 H, 8-OH), 8.34 (s, 1 H, 3-H), 8.29 (s, 1 H, 2-NH), 6.34 (d, *J* = 12.6 Hz, 1 H, 29-H), 6.28 (dd, *J* = 15.7, 8.3 Hz, 1 H, 18-H), 5.86 (d, *J* = 8.3 Hz, 1 H, 17-H), 5.77 (dd, *J* = 15.7, 8.3 Hz, 1 H, 19-H), 5.27 (dd, *J* = 12.6, 8.6 Hz, 1 H, 28-H), 4.63 (d, *J* = 1.5 Hz, 1 H, 25-H), 3.91 (d, *J* = 4.2 Hz, 1 H, 23-OH), 3.71 (s, 1 H, 21-OH), 3.49 (d, *J* = 9.5 Hz, 1 H, 21-H), 3.01 (dd, *J* = 9.0, 3.4 Hz, 1 H, 27-H), 2.96 (ddd, *J* = 10.1, 4.3, 1.7 Hz, 1 H, 23-H), 2.78 (s, 3 H, 37-H), 2.37 s, 3 H, 14-H), 2.28 – 2.24 (m, 1 H, 20-H), 1.90 – 1.87 (m, 1 H, 26-H), 1.79 (s, 3 H, 30-H), 1.60 (s, 3 H, 13-H), 1.59

(s, 3 H, 36-H), 1.50 (m, 1 H, 22-H), 1.47 (m, 1 H, 24-H), 0.99 (d, *J* = 7.0 Hz, 3 H, 32-H), 0.64 (d, *J* = 7.3 Hz, 3 H, 31-H), 0.45 (d, *J* = 7.1 Hz, 3 H, 33-H), 0.23 (d, *J* = 7.1 Hz, 3 H, 34-H); **^{13}C NMR** (125 MHz, C_6D_6, C_6D_6 = 128.06 ppm) δ (ppm): 190.7 (s, C-11), 185.4 (s, C-1), 181.8 (s, C-4), 173.5 (s, C-35), 172.5 (s, C-6), 169.2 (s, C-15), 167.0 (s, C-8), 146.3 (d, C-29), 142.5 (d, C-19), 139.8 (s, C-2), 133.1 (d, C-17), 131.5 (s, C-16), 131.5 (s , C-10), 124.7 (d, C-18), 117.1 (d, C-3), 115.2 (d, C-28), 115.1 (s, C-7), 111.5 (s, C-5), 111.4 (s, C-9), 109.1 (s, C-12), 82.8 (d, C-27), 78.0 (d, C-23), 73.9 (d, C-21), 73.9 (d, C-25), 56.3 (q, C-37), 40.4 (d, C-20), 37.7 (d, C-26), 37.4 (d, C-24), 33.1 (d, C-22), 22.8 (q, C-13), 20.6 (q, C-36), 20.3 (q, C-30), 16.6 (q, C-31), 12.4 (q, C-34), 11.8 (q, C-32), 8.9 (q, C-33), 7.5 (q, C-14); **UPLC-MS** (MeOH) t_R = 3.0 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{37}H_{46}NO_{12}]^+$: 696.3020, found: 696.3022.

Rifamycin W (24)



Rifamycin W (**24**) was obtained as a yellow solid after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-amino-5-hydroxybenzoic acid (**7**) (fed 50 mg – 0.33 mmol/125 mL; 10 mg/plate) carried out on agar plates (isolated amount = 1.6 mg; 12.8 mg/L).

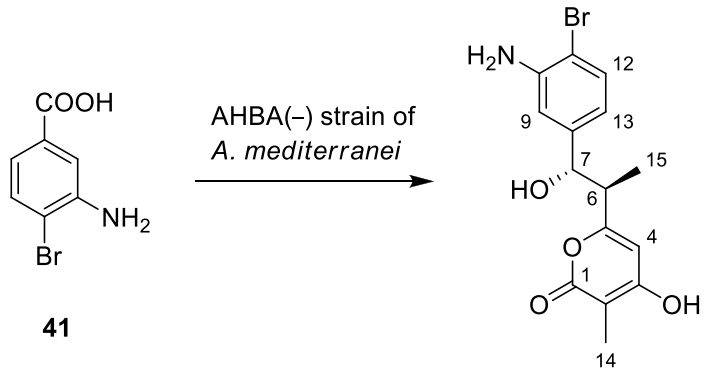
1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 67.0 min.

2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 70/55; 90/100; 100/100; t_R = 74.0 min.

The analytical data are in accordance with the reported in the literature.[30,31]

^{1}H NMR (500 MHz, CD_3OD, CHD_2OD = 4.87 ppm) δ (ppm): 8.40 (bs, 1H, 2-NH), 7.57 (s, 1H, 3-H), 6.50 (dd, *J* = 16.0, 11.0 Hz, 1H, 18-H), 6.41 (d, *J* = 10.0 Hz, 1H, 29-H), 6.25 (d, *J* = 11.0 Hz, 1H, 17-H), 6.08 (dd, *J* = 16.0, 6.7 Hz, 1H, 19-H), 4.61 (bs, 1H, 6-OH), 4.39 (bs, 1H, 8-OH), 4.04 (dd, *J* = 10.0, 1.0 Hz, 1H, 21-H), 4.01 (dd, *J* = 10.0, 1.0 Hz, 1H, 23-H), 3.58 (dd, *J* = 10.9, 7.9 Hz, 1H, 34aa-H), 3.48 (dd, *J* = 10.0, 2.0 Hz, 1H, 25-H), 3.44 (dd, *J* = 10.8, 6.5 Hz, 1H, 34ab-H), 3.21 – 3.13 (m, 1H, 28-H), 2.63 (dd, *J* = 16.6, 7.3 Hz, 1H, 27-H), 2.38 – 2.32 (m, 1H, 20-H), 2.16 (s, 3H, 14-H), 2.09 (bs, 3H, 13-H), 2.08 (bs, 3H, 30-H), 1.88 – 1.85 (m, 1H, 22-H), 1.82 – 1.79 (m, 1H, 24-H), 1.44 – 1.38 (m, 1H, 26-H), 1.06 (d, *J* = 7.1 Hz, 3H, 33-H or 34-H), 0.92 (d, *J* = 7.0 Hz, 3H, 31-H), 0.73 (d, *J* = 6.8 Hz, 3H, 33-H or 34-H), 0.43 (d, *J* = 7.0 Hz, 3H, 32-H); **^{13}C NMR** (125 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 201.2 (s, C-11), 187.1 (s, C-4), 183.4 (s, C-1), 172.2 (s, C-15), 164.7 (s, C-8), 164.1 (s, C-6), 142.6 (d, C-19), 141.5 (d, C-29), 140.9 (s, C-12), 140.8 (s, C-2), 135.2 (d, C-17), 132.1 (s, C-16), 129.9 (s, C-10), 126.3 (d, C-18), 122.2 (s, C-5), 119.0 (s, C-7), 118.0 (d, C-3), 107.5 (s, C-9), 79.0 (d, C-27), 74.8 (d, C-23), 71.0 (d, C-21), 69.1 (d, C-25), 64.5 (t, C-34a), 49.6 (d, C-28), 44.1 (d, C-26), 39.1 (d, C-20), 37.9 (d, C-24), 34.3 (d, C-22), 20.2 (q, C-30), 18.0 (q, C-13), 12.7 (q, C-34), 11.7 (q, C-32), 11.2 (q, C-31), 8.9 (q, C-33), 8.5 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.9 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{35}H_{46}NO_{11}]^+$: 656.3071, found: 656.3068.

Tetraketide 42



41 **42**

Tetraketide **42** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-amino-4-bromobenzoic acid (**41**) (fed 214 mg – 0.99 mmol/800 mL). The fermentation was carried out in liquid medium providing compound **42** as a colorless solid (isolated amount = 15.0 mg; 19.0 mg/L). The yield is better than the one

obtained after fermentation on agar plates (fed 300 mg – 0. 94 mmol/750 mL; 10 mg/plate; isolated amount = 7.0 mg/750 mL; 9.0 mg/L).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 50.0 min.

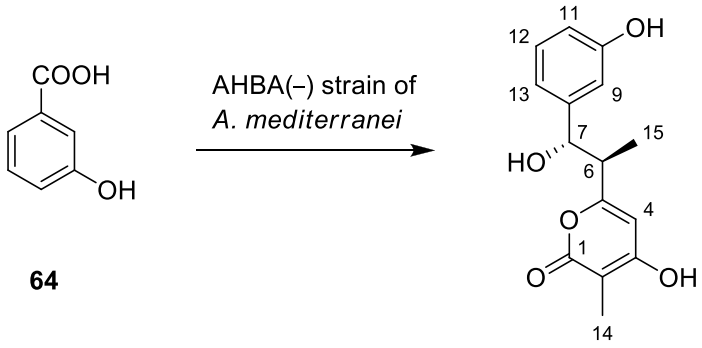
2. HPLC: preparative HPLC (C18-1): solvent A: water, solvent B: MeOH; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 50/55; 90/100; 100/100; t_R = 56.0 min.

3. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 80/45; 90/100; 100/100; t_R = 64.0 min.

The analytical data are in accordance with the reported in the literature.[68]

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 7.33 (d, *J* = 8.2 Hz, 1H, H-12), 6.82 (d, *J* = 2.0 Hz, 1H, H-9), 6.54 (dd, *J* = 8.2, 2.0 Hz, 1H, H-13), 6.04 (s, 1H, H-4), 4.63 (d, *J* = 8.6 Hz, 1H, H-7), 2.81 (dq, *J* = 14.1, 7.0 Hz, 1H, H-6), 1.87 (s, 3H, H-14), 0.99 (d, *J* = 7.1 Hz, 3H, H-15); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 169.2 (s, C-1), 168.2 (s, C-3), 165.8 (s, C-5), 146.5 (s, C-10), 144.3 (s, C-8), 133.3 (d, C-12), 118.1 (d, C-13), 115.4 (d, C-9), 108.9 (s, C-11), 102.4 (d, C-4), 99.2 (s, C-2), 76.6 (d, C-7), 47.2 (d, C-6), 15.5 (q, C-15), 8.3 (q, c-14); **UPLC-MS** (MeOH) t_R = 2.6 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{17}BrNO_4]^+$: 354.0341, found: 354.0341.

Tetraketide 67



64 **67**

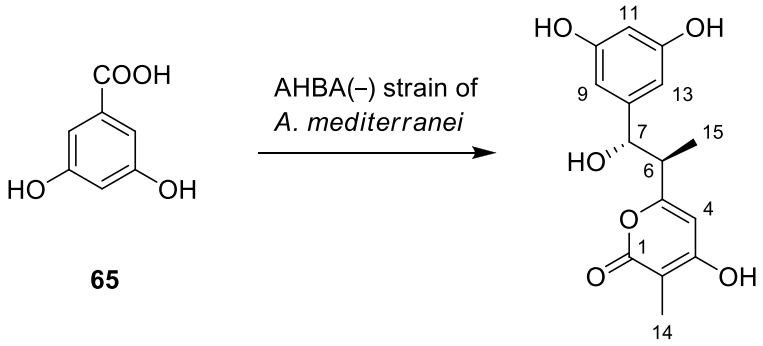
Tetraketide **67** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-hydroxybenzoic acid (**64**) (fed 173 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **67** as a colorless foam (isolated amount = 118.0 mg).

HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 44.0 min.

The analytical data are in accordance with the reported in the literature.[75]

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 7.15 (t, *J* = 8.1 Hz, 1H, H-12), 6.82 – 6.76 (m, 2H, H-11 or H-13, H-9), 6.73 – 6.66 (m, 1H, H-11 or H-13), 6.06 (s, 1H, H-4), 4.67 (d, *J* = 8.8 Hz, 1H, H-7), 2.83 (dq, *J* = 14.1, 7.1 Hz, 1H, H-6), 1.88 (s, 3H, H-14), 0.98 (d, *J* = 7.0 Hz, 3H, H-15); **^{1}H NMR** (400 MHz, THF-d_8, THF-d_7 = 1.73 ppm) δ (ppm): 7.06 (t, *J* = 7.8 Hz, 1H, H-12), 6.75 – 6.73 (m, 2H, H-11 or H-13, H-9), 6.62 (ddd, *J* = 8.0, 2.3, 1.0 Hz, 1H, H-11 or H-13), 5.85 (s, 1H, H-4), 4.64 (d, *J* = 8.4 Hz, 1H, H-7), 2.71 (dq, *J* = 15.0, 7.1 Hz, 1H, H-6), 1.83 (s, 3H, H-14), 0.93 (d, *J* = 7.1 Hz, 3H, H-15); **^{13}C NMR** (100 MHz, THF-d_8, THF-d_8 = 25.5 ppm) δ (ppm): 166.0 (s, C-3), 165.4 (s, C-5), 165.2 (s, C-1), 158.6 (s, C-10), 146.0 (s, C-8), 129.6 (d, C-12), 118.6 (d, C-13), 115.2 (d, C-11), 114.6 (d, C-9), 100.9 (s, C-2),98.7 (d, C-4), 76.2 (d, C-7), 47.4 (d, C-6), 15.3 (q, C-15), 8.7 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.4 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{17}O_5]^+$: 277.1076, found: 277.1075; **$[\alpha]^{20}_D$** = −14.3° (*c* = 1.0, MeOH).

Tetraketide 68



65 **68**

Tetraketide **68** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3,5-dihydroxybenzoic acid (**65**) (fed 193 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **68** as a colorless solid (isolated amount = 4.2 mg).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 41.0 min.

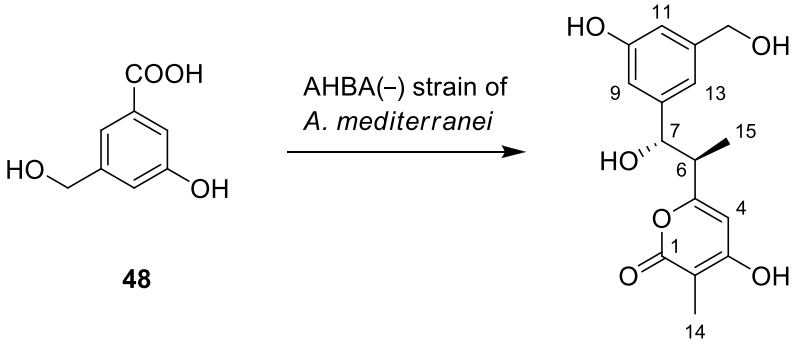
2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 80/45; 90/100; 100/100; t_R = 32.0 min.

3. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 60/25; 90/90, 100/100; t_R = 37.0 min.

The analytical data are in accordance with the reported in the literature.[75]

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 6.29 (d, *J* = 2.2 Hz, 2H, H-9, H-13), 6.19 (t, *J* = 2.2 Hz, 1H, H-11), 6.04 (s, 1H, H-4), 4.58 (d, *J* = 8.8 Hz, 1H, H-7), 2.78 (dq, *J* = 14.2, 7.1 Hz, 1H, H-6), 1.87 (s, 3H, H-14), 0.99 (d, *J* = 7.1 Hz, 3H, H-15); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 169.4 (s, C-3), 169.0 (s, C-5), 165.9 (s, C-1), 159.6 (s, C-10, C-12), 146.1 (s, C-8), 106.4 (d, C-9, C-13), 103.0 (d, C-11), 102.7 (s, C-4), 99.0 (d, C-2), 77.0 (d, C-7), 47.2 (d, C-6), 15.6 (q, C-15), 8.34 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.2 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{17}O_6]^+$: 293.1025, found: 293.1028; $\mathbf{[\alpha]^{20}_D}$ = −3.0° (*c* = 0.33, MeOH).

Tetraketide 69



48 **69**

Tetraketide **69** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-hydroxy-5-(hydroxymethyl)benzoic acid (**48**) (fed 210 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **69** as a colorless solid which gradually decomposed during HPLC purification steps.

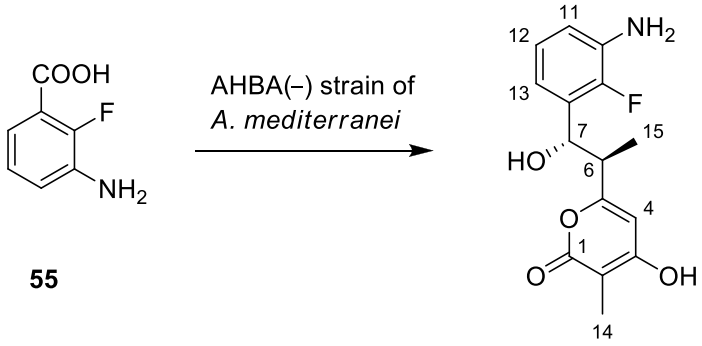
1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 38.0 min.

2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 80/45; 90/100; 100/100; t_R = 30.0 min.

3. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 60/25; 90/90, 100/100; t_R = 47.0 min.

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 6.81 (s, 1H, Ar), 6.74 (s, 1H, Ar), 6.68 (s, 1H, Ar), 6.04 (s, 1H, H-4), 4.67 (d, *J* = 8.8 Hz, 1H, H-7), 4.53 (s, 2H, $ArCH_2$), 2.82 (dq, *J* = 14.2, 7.1 Hz, 1H, H-6), 1.86 (s, 3H, H-14), 0.97 (d, *J* = 7.0 Hz, 3H, H-15); **UPLC-MS** (MeOH) t_R = 2.0 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{16}H_{19}O_6]^+$: 307.1182, found: 307.1187.

Tetraketide 87



55 **87**

Tetraketide **87** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-amino-2-fluorobenzoic acid (**55**) (fed 194 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **87** as a colorless solid (isolated amount = 25.0 mg).

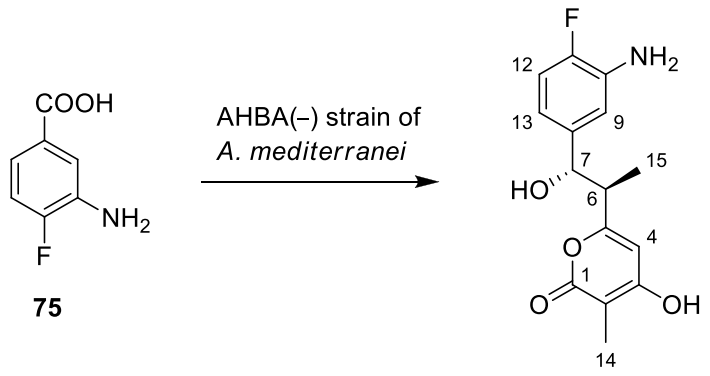
1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 39.0 min.

2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/5; 10/5; 20/10; 60/40; 90/100; 100/100; t_R = 46.0 min.

3. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 10/10; 20/10; 60/40; 100/100; t_R = 68.0 min.

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 6.90 (t, J = 7.8 Hz, 1H, H-12), 6.78 – 6.71 (m, 2H, H-13, H-11), 6.06 (s, 1H, H-4), 5.09 (d, J = 8.9 Hz, 1H, H-7), 2.90 (dq, J = 14.4, 7.2 Hz, 1H, H-6), 1.86 (s, 3H, H-14), 1.01 (d, J = 7.0 Hz, 3H, H-15); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 169.2 (s, C-3), 168.2 (s, C-5), 165.7 (s, C-1), 150.7 (s, C-9), 136.7 (s, C-10), 130.5 (s, C-8), 125.3 (d, C-12), 117.4 (d, C-13), 117.2 (d, C-11), 102.5 (d, C-4), 99.2 (s, C-2), 70.3 (d, C-7), 46.8 (d, C-6), 15.3 (q, C-15), 8.3 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.3 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{17}FNO_4]^+$: 294.1142, found: 294.1140; $\mathbf{[\alpha]^{20}_D}$ = −11.2° (*c* = 1.15, MeOH).

Tetraketide 88



75 **88**

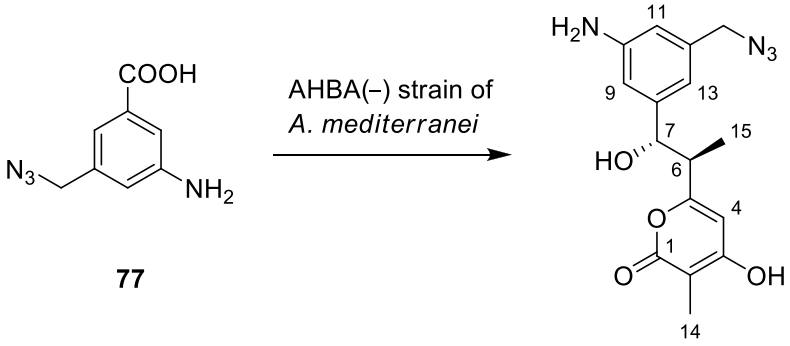
Tetraketide **88** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-amino-4-fluorobenzoic acid (**75**) (fed 194 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **88** as a colorless solid (isolated amount = 20 mg).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 46.0 min.

2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/5; 10/5; 20/10; 60/40; 90/100; 100/100; t_R = 50.0 min.

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 6.90 (dd, *J* = 11.2, 8.3 Hz, 1H, H-12), 6.83 (dd, *J* = 8.7, 2.1 Hz, 1H, H-9), 6.60 (ddd, *J* = 8.3, 4.4, 2.1 Hz, 1H, H-13), 6.04 (s, 1H, H-4), 4.62 (d, *J* = 8.8 Hz, 1H, H-7), 2.79 (dq, *J* = 14.1, 7.1 Hz, 1H, H-6), 1.86 (s, 3H, H-14), 0.95 (d, *J* = 7.0 Hz, 3H, H-15); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 169.2 (s, C-3), 168.1 (s, C-5), 166.0 (s, C-1), 152.7 (s, C-11), 140.1 (s, C-8), 136.7 (s, C-10), 117.4 (d, C-13), 116.7 (d, C-9), 115.6 (d, C-12), 102.3 (d, C-4), 99.2 (s, C-2), 76.6 (d, C-7), 47.3 (d, C-6), 15.6 (q, C-15), 8.3 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.4 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{17}FNO_4]^+$: 294.1142, found: 294.1144; $\mathbf{[\alpha]^{20}_D}$ = −19.3° (*c* = 0.75, MeOH).

Tetraketide 89



77 **89**

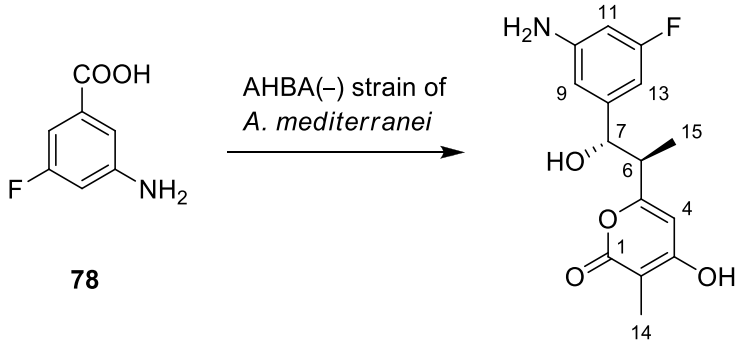
Tetraketide **89** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-amino-5-(azidomethyl)benzoic acid (**77**) (fed 240 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **89** as a colorless solid (isolated amount = 2.5 mg).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 36.0 min.

2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 50/35; 90/80; 100/100; t_R = 49.0 min.

^{1}H NMR (500 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 6.69 (t, *J* = 1.6 Hz, 1H, H-13), 6.65 – 6.60 (m, 2H, H-9, H-11), 6.04 (s, 1H, H-4), 4.64 (d, *J* = 8.8 Hz, H-7), 4.21 (s, 2H, ArCH_2), 2.81 (dq, *J* = 14.1, 7.1 Hz, H-6), 1.86 (s, 3H, H-14), 0.97 (d, *J* = 7.1 Hz, H-15); **^{13}C NMR** (125 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 169.4 (s, C-3), 168.9 (s, C-5), 165.9 (s, C-1), 149.6 (s, C-10), 145.4 (s, C-8), 137.9 (s, C-12), 117.5 (d, C-13), 115.7 (d, C-11), 114.8 (d, C-9), 102.7 (d, C-4), 99.1 (s, C-2), 77.0 (d, C-7), 55.7 (t, ArCH_2), 47.3 (d, C-6), 15.6 (q, C-15), 8.3 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.54 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{16}H_{19}N_4O_4]^+$: 331.1406, found: 331.1406; $\mathbf{[\alpha]^{20}_D}$ = −4.3° (*c* = 0.2, MeOH).

Tetraketide 90



78 **90**

Tetraketide **90** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-amino-5-fluorobenzoic acid hydrochloride (**78**·HCl) (fed 239 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **90** as a colorless solid (isolated amount = 2.5 mg).

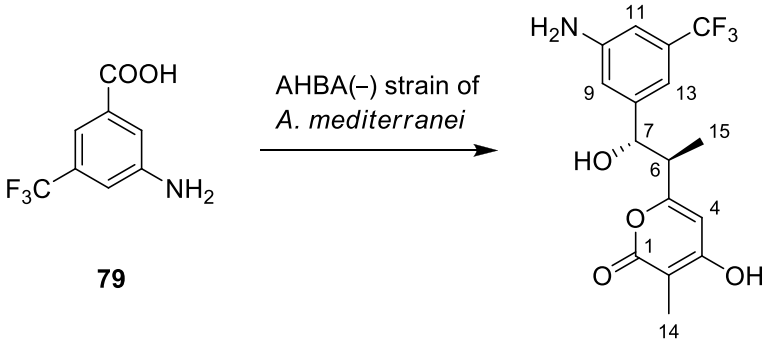
1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 39.0 min.

2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 80/45; 90/100; 100/100; t_R = 62.0 min.

3. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 10/10; 20/30; 60/40; 90/90, 100/100; t_R = 43.0 min.

^{1}H NMR (500 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 6.45 (t, J = 2.0 Hz, 1H, H-9), 6.35 – 6.32 (dt, *J* = 11.0, 2.0 Hz 1H, H-13), 6.31 – 6.29 (dt, *J* = 11.0, 2.0 Hz, 1H, H-11), 6.04 (s, 1H, H-4), 4.59 (d, *J* = 8.7 Hz, H-7), 2.77 (dq, *J* = 14.0, 7.1 Hz, H-6), 1.85 (s, 3H, H-14), 0.98 (d, *J* = 7.1 Hz, H-15); **^{13}C NMR** (125 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 169.3 (s, C-3), 168.8 (s, C-5), 165.7 (s, C-1), 165.3 (s, C-12), 146.9 (s, C-10), 146.8 (s, C-8), 110.5 (d, C-9), 103.0 (d, C-13), 102.7 (s, C-2), 101.8 (d, C-11), 99.1 (d, C-4), 76.7 (d, C-7), 47.1 (d, C-6), 15.6 (q, C-15), 8.3 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.3 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{17}FNO_4]^+$: 294.1142, found: 294.1140.

Tetraketide 91



79 **91**

Tetraketide **91** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-amino-5-(trifluoromethyl)benzoic acid (**79**) (fed 256 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **91** as a colorless solid (isolated amount = 1.0 mg).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 57.0 min.

2. HPLC: preparative HPLC (C18-1): solvent A: water, solvent B: MeOH; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 50/35; 90/100; 100/100; t_R = 74.0 min.

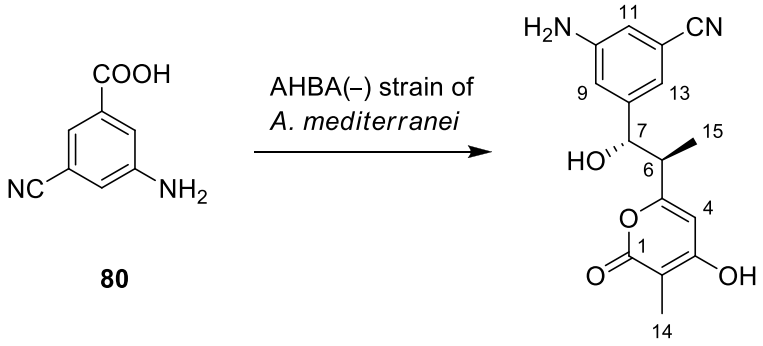
3. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 68.0 min.

4. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]):0/10; 5/10; 50/35; 90/100; 100/100; t_R = 69.0 min.

^{1}H NMR (500 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 6.92 – 6.86 (m, 3H, H-13, H-11, H-9), 6.01 (s, 1H, H-4), 4.72 (d, *J* = 8.5 Hz, 1H, H-7), 2.81 (dq, *J* = 14.0, 6.9 Hz, 1H, H-6), 1.87 (s, 3H, H-14), 1.00 (d, *J* = 7.1 Hz, 3H, H-15); **^{13}C NMR** (125 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 179.4 (s, C-3), 169.3 (s, C-5), 165.5 (s, C-1), 150.2 (s, C-10), 146.0 (s, C-8), 132.4 (s, C-12), 125.8 (s, C-16), 117.6 (d, C-9), 112.8 (d, C-13), 111.3 (d, C-11), 102.7 (d, C-4), 99.2 (s, C-2), 76.6 (d, C-7), 47.1 (d, C-6), 15.3 (q, C-15), 8.3 (q, C-14);

UPLC-MS (MeOH) t_R = 2.6 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{16}H_{17}F_3NO_4]^+$: 344.1110, found: 344.1114.

Tetraketide 92



80 **92**

Tetraketide **92** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-amino-5-cyanobenzoic acid (**80**) (fed 102 mg – 0.63 mmol/500 mL). The fermentation was carried out in liquid medium providing compound **92** as a colorless solid (isolated amount = 3.5 mg; 7.0 mg/L).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 90/100; 100/100; t_R = 41.0 min.

2. HPLC: preparative HPLC (C18-1): solvent A: water, solvent B: MeOH; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 70/50; 90/100; 100/100; t_R = 51.0 min.

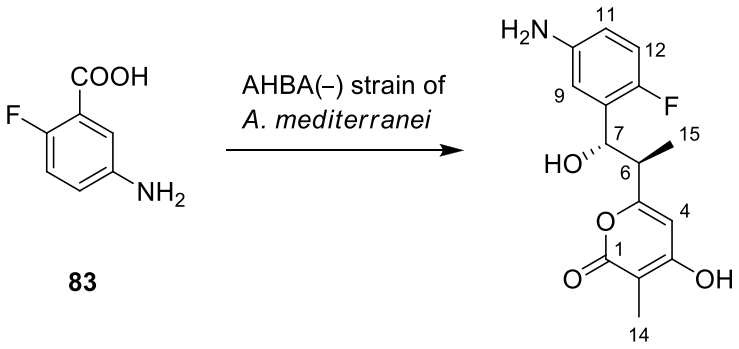
3. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 70/50; 90/100; 100/100; t_R = 21.0 min.

3. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/5; 5/5; 50/5; 90/100; 100/100; t_R = 65.0 min.

^{1}H NMR (500 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 6.90 – 6.86 (m, 2H, H-11, H-9), 6.84 (dd, *J* = 2.1. 1.5 Hz, 1H, H-3), 6.04 (s, 1H, H-4), 4.65 (d, *J* = 8.5 Hz, 1H, H-7), 2.81 (dq, *J* = 14.2, 7.1 Hz, 1H, H-6), 1.86 (s, 3H, H-14), 0.99 (d, *J* = 7.1 Hz, 3H, H-15); **^{13}C NMR** (125 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 169.3 (s, C-3), 168.3 (s, C-5), 165.6 (s, C-1), 150.7 (s, C-10), 146.7 (s, C-8), 120.5 (s, C-16), 119.8 (d, C-11), 118.8 (d, C-9),

117.4 (d, C-13), 113.7 (s, C-12), 102.7 (d, C-4), 99.5 (s, C-2), 76.4 (d, C-7), 47.2 (d, C-6), 15.6 (q, C-15), 8.5 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.1 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{16}H_{17}N_2O_4]^+$: 301.1188, found: 301.1140.

Tetraketide 93



83 **93**

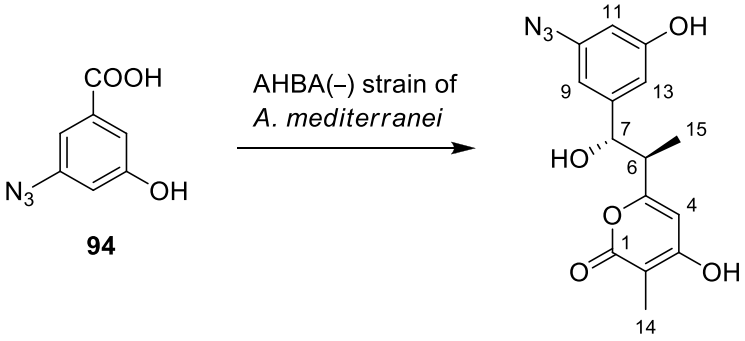
Tetraketide **93** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 5-amino-2-fluorobenzoic acid (**83**) (fed 194 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **93** as a colorless solid (isolated amount = 1.1 mg).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 26.0 min.

2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5/10; 80/45; 90/100; 100/100; t_R = 50.0 min.

^{1}H NMR (500 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 8.25 (s, 1H, 7-OH), 7.64 (dd, *J* = 6.3, 2.6 Hz, H-12), 7.61 – 7.57 (m, 1H, H-9), 7.09 – 7.02 (m, 1H, H-11), 5.95 (s, 1H, H-4), 5.15 (d, *J* = 8.4 Hz, 1H, H-7), 2.89 (dq, *J* = 14.5, 7.0 Hz, 1H, H-6), 1.85 (s, 3H, H-14) 1.06 (d, *J* = 7.0 Hz, H-15); **^{13}C NMR** (125 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 164.2 (s, C-3), 163.7 (C-5) 161.5 (s, C-1), 159.4 (s, C-13), 135.4 (s, C-10), 131.7 (s, C-8), 122.3 (d, C-11), 121.0 (d, C-9), 117.0 (s, C-2), 116.4 (d, C-12), 98.0 (d, C-4), 69.8 (d, C-7), 46.8 (d, C-6), 15.1 (q, C-15), 8.6 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.2 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{17}FNO_4]^+$: 294.1142, found: 294.1130; $\boldsymbol{[\alpha]^{20}_D}$ = −9.0° (*c* = 0.07, MeOH).

Tetraketide 96



94

96

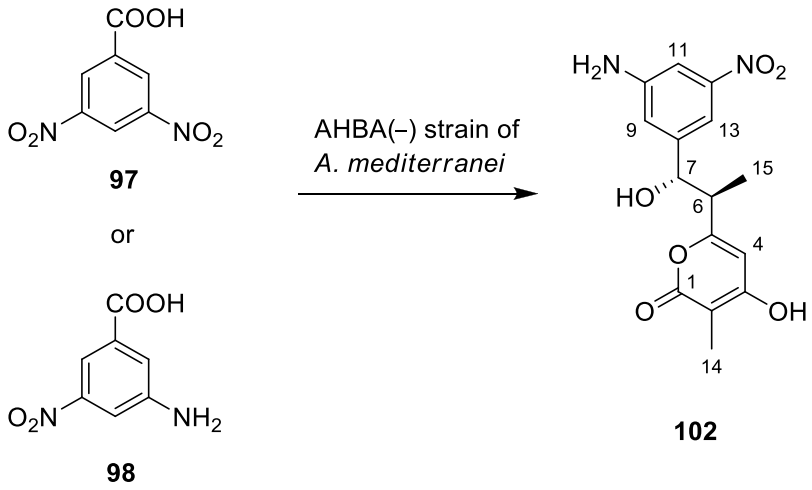
Tetraketide **96** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3-azido-5-hydroxybenzoic acid (**94**) (fed 168 mg – 0.94 mmol/750 mL). The fermentation was carried out in liquid medium providing compound **96** as brown crystals (isolated amount = 35 mg; 47 mg/L).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 53.0 min.

2. HPLC: preparative HPLC (C18-1): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10; 5.0/10; 50/55; 90/100; 100/100; t_R = 55.0 min.

^{1}H NMR (400 MHz, CD_3OD, CHD_2OD = 3.31 ppm) δ (ppm): 8.06 (s, 1H, 12-OH), 6.54 – 6.51 (m, 1H, 9-H), 6.47 – 6.44 (m, 1H, 13-H), 6.33 (t, *J* = 2.1 Hz, 1H, 11-H), 6.01 (s, 1H, 4-H), 4.62 (d, *J* = 8.4 Hz, 1H, 7-H), 3.89 (s, 1H, 7-OH), 2.76 (dq, *J* = 8.3, 7.1 Hz, 1H, 6-H), 1.81 (s, 3H, 14-H), 0.95 (d, *J* = 7.1 Hz, 3H, 15-H); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 169.1 (s, C-3), 167.8 (s, C-5), 165.6 (s, C-1), 159.9 (s, C-12), 147.1 (s, C-10), 142.41 (s, C-8), 112.0 (d, C-9), 109.3 (d, C-11), 106.3 (d, C-13), 102.3 (s, C-2), 99.3 (d, C-4), 76.5 (d, C-7), 47.1 (d, C-6), 15.3 (q, C-15), 8.3 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.3 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{16}N_3O_5]^+$: 318.1090, found: 318.1080; $\boldsymbol{[\alpha]^{20}_D}$ = +7.8° (*c* = 0.5, MeOH).

Tetraketide 102



Tetraketide **102** was obtained after supplementing a culture of the blocked mutant of *A. mediterranei* (strain HGF003) with 3,5-dinitrobenzoic acid (**97**) (fed 27 mg – 0.12 mmol/100 mL) and 3-amino-5-nitrobenzoic acid (**98**) (fed 57 mg – 0.31 mmol/250 mL). The fermentations were carried out in liquid medium providing compound **102** as a yellow solid (isolated amount = 7.0 mg; 70 mg/L and 18.0 mg; 72 mg/L, respectively).

Fermentation with 3,5-dinitrobenzoic acid (**97**) (fed 50 mg – 0. 24 mmol/125 mL; 10 mg/plate) carried out on agar plates provided 2.0 mg of the title compound **102** (16 mg/L).

1. HPLC: semi-preparative HPLC (C18-2): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5.0/20; 50/55; 90/100; 100/100; t_R = 18.0 min.

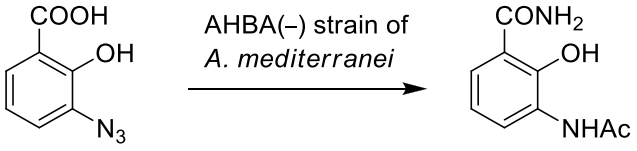
2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/15; 5.0/15; 80/45; 90/100; 100/100; t_R = 33.0 min.

3. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/5; 5.0/5; 15.0/5; 90/100; 100/100; t_R = 68.0 min.

^{1}H NMR (400 MHz, CD_3OD, CHD_2OD = 3.31 ppm) δ (ppm): 7.51 − 7.43 (m, 1H, H-13), 7.40 (t, *J* = 2.2 Hz, 1H, H-11), 7.00 − 6.94 (m, 1H, H-9), 6.05 (s, 1H, H-4), 4.76 (d, *J* = 8.3 Hz, 1H, H-7), 4.59 (bs, 1H, OH), 2.86 (dq, *J* = 8.3, 7.1 Hz, 1H, H-6), 1.87 (s, 3H, H-14), 1.03 (d, *J* = 7.1 Hz, 3H, H-15); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 171.4

(s, C-3), 169.8 (s, C-5), 164.7 (s, C-1), 150.9 (s, C-12), 150.7 (s, C-10), 146.6 (s, C-8), 119.8 (d, C-11), 110.6 (d, C-13), 109.3 (d, C-4), 108.5 (d, C-9), 98.5 (s, C-2), 76.2 (d, C-7), 47.0 (d, C-6), 15.2 (q, C-15), 8.5 (q, C-14); **UPLC-MS** (MeOH) t_R = 2.4 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{17}N_2O_6]^+$: 321.1087, found: 354.1065.

3-Acetamido-2-hydroxybenzamide (103)



63 **103**

3-Acetamido-2-hydroxybenzamide (**103**) was obtained after feeding to the blocked mutant of *A. mediterranei* HGF003 3-azido-2-hydroxybenzoic acid (**63**) (fed 224 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **103** as a colorless solid (isolated amount = 11.1 mg).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 23.0 min.

2. HPLC: preparative HPLC (C18-1): solvent A: water, solvent B: MeOH; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/10, 20/15, 60/25, 90/45, 100/100; t_R = 41.0 min.

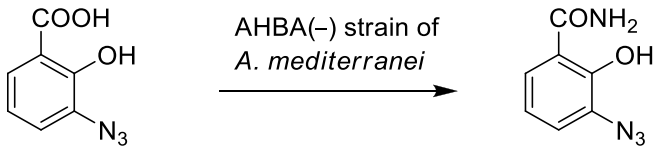
3. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10, 20/15, 60/15, 90/45, 100/100; t_R = 7.0 min.

4. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/5, 20/10, 60/10, 90/45, 100/100; t_R = 7.0 min.

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.5 ppm) δ (ppm): 13.94 (s, 1H, OH), 9.21 (s, 1H, NHAc), 8.67 (s, 1H, $CONH_2$), 8.07 (dd, *J* = 7.8, 1.0 Hz, 1H, H-6), 7.93 (s, 1H, $CONH_2$), 7.56 (dd, *J* = 8.0, 1.2 Hz, 1H, H-4), 6.75 (t, *J* = 8.0 Hz, 1H, H-5); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 174.5 (s, $CONH_2$), 171.9 (s, NH*C*OCH_3), 154.2 (s, C-2), 128.6 (s, C-3), 127.0 (d, C-4), 124.1 (d, C-6), 118.7 (d, C-5), 115.3 (s, C-1), 23.8 (q, NHCO*C*H_3); **UPLC-MS** (MeOH) t_R = 1.9 min; **HRMS-ESI** (*m*/*z*):

$[M+H]^+$ calcd for $[C_9H_{11}N_2O_3]^+$: 195.0770, found: 195.0768; $[M-H]^-$ calcd for $[C_9H_9N_2O_3]^-$: 193.0619, found: 193.0612.

3-Azido-2-hydroxybenzamide (104)



63 **104**

3-Azido-2-hydroxybenzamide (**104**) was obtained after feeding to the blocked mutant of *A. mediterranei* HGF003 3-azido-2-hydroxybenzoic acid (**63**) (fed 224 mg – 1.25 mmol/L). The fermentation was carried out in liquid medium providing compound **104** as a brown solid (isolated amount = 7.7 mg).

1. HPLC: preparative HPLC (C18-1): solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 50/55; 90/100; 100/100; t_R = 44.0 min.

2. HPLC: semi-preparative HPLC (C18-2): solvent A: water, solvent B: MeOH; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/10, 20/15, 60/25, 90/45, 100/100; t_R = 27.0 min.

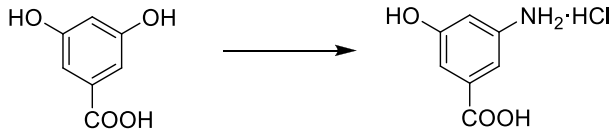
^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.5 ppm) δ (ppm): 14.11 (s, 1H, OH), 8.67 (s, 1H, $CONH_2$), 8.10 (s, 1H, $CONH_2$), 7.67 (dd, *J* = 8.0, 1.3 Hz, 1H, H-6), 7.15 (dd, *J* = 7.6, 1.0 Hz, 1H, H-4), 6.82 (t, *J* = 7.9 Hz, H-5); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 174.2 (s, $CONH_2$), 156.3 (s, C-2), 129.4 (s, C-3), 125.7 (d, C-4), 125.2 (d, C-5), 119.6 (d, C-6), 116.4 (s, C-1); **UPLC-MS** (MeOH) t_R = 1.92 min; **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_7H_7N_4O_2]^+$: 179.0569, found: 179.0560; $[M-H]^-$ calcd for $[C_7H_5N_4O_2]^-$: 177.0418, found: 177.0416.

3.4 Synthesis of Mutasynthons

The preparation of the benzoic acid derivatives was carried out according to the literature procedures: 3-amino-4-fluorobenzoic acid (**75**),[73,160] 3-amino-5-(azidomethyl)benzoic acid (**77**),[57] 3-amino-5-cyanobenzoic acid (**80**),[161] 3-amino-5-(hydroxymethyl)benzoic acid (**82**),[70] 3-azido-5-hydroxybenzoic acid (**94**).[57]

Mutasynthons **41** (3-amino-4-bromobenzoic acid), **64** (3-hydroxybenzoic acid), **65** (3,5-dihydroxybenzoic acid), **70** (anthranilic acid), **71** (2-amino-3-nitrobenzoic acid), **72** (2-amino-4-nitrobenzoic acid), **73** (4-aminobenzoic acid), **74** (4-amino-3-nitrobenzoic acid), **79** (3-amino-5-(trifluoromethyl)benzoic acid), **81** (3-amino-5-(methoxycarbonyl)benzoic acid), **84** (5-amino-2-nitrobenzoic acid), **85** (3-amino-2,5,6-trifluorobenzoic acid), **86** (2,5-dihydroxybenzoic acid), **95** (3-hydroxy-5-nitrobenzoic acid), **97** (3,5-dinitrobenzoic acid), **98** (3-amino-5-nitrobenzoic acid) are commercially available.

3-Amino-5-hydroxybenzoic acid hydrochloride (7·HCl**)**



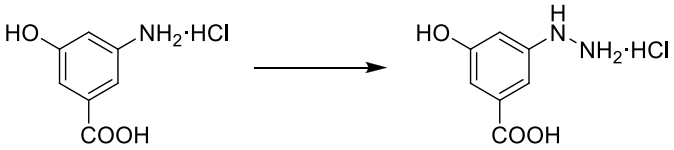
65 **7**

3,5-Dihydroxybenzoic acid (**65**) (22.5 g, 146 mmol, 1.0 eq.), ammonium chloride (19.1 g, 358 mmol, 2.45 eq.) and conc. NH_3 (30% in water, 67.5 mL) were placed in a steel bomb (model T304, PARR INSTRUMENT COMPANY) and heated at 180 °C for 40 h. After cooling down the reaction mixture was evaporated *in vacuo* to dryness and the residue was treated with 6 M HCl (50 mL) followed by heating under reflux for 3 h. After crystallization at 4 °C the brownish solid was recrystallized upon addition of activated charcoal from half-conc. HCl. The desired hydrochloride **7**·HCl (15.5 g, 81.8 mmol, 56%) was obtained as a crystalline, beige solid.

The analytical data are in accordance with the reported in the literature.[138,139]

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 7.51 (dd, J = 2.3, 1.3 Hz, 1H, H-6), 7.49 (dd, J = 2.0, 1.4 Hz, 1H, H-2), 7.07 (t, J = 2.2 Hz, 1H, H-4), 5.04 (s, NH_3); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 168.0 (s, COOH), 160.4 (s, C-5), 135.1 (s, C-3), 133.1 (s, C-1), 118.1 (d, C-4), 115.7 (d, C-6), 115.4 (d, C-2); **HRMS-ESI** (*m*/*z*): $[M-H]^-$ calcd for $[C_7H_6NO_3]^-$: 152.0348, found: 152.0351; $[M+H]^+$ calcd for $[C_7H_8NO_3]^+$: 154.0504, found: 154.0507; **M.p.**: 200–203 °C (Lit. 199–200 °C).[139]

2-(3-Carboxy-5-hydroxyphenyl)-hydrazin-1-ium chloride (66·HCl)



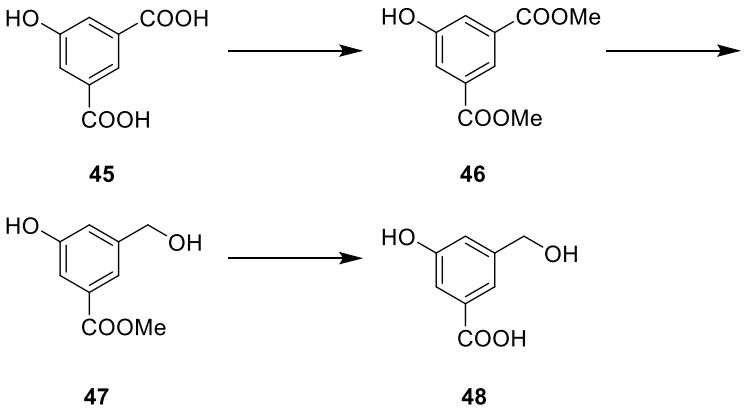
7 **66**

3-Amino-5-hydroxybenzoic acid hydrochloride (**7**·HCl) (0.1 g, 0.53 mmol, 1.0 eq.) was suspended in conc. HCl (5.0 mL) and cooled down to −10 °C. $NaNO_2$ (0.06 g, 0.8 mmol, 1.5 eq.) was dissolved in water (0.5 mL) and added to the substrate. After 30 minutes $SnCl_2 \cdot H_2O$ (0.36 g, 1.58 mmol, 3.0 eq.) in conc. HCl (0.5 mL) was added and the reaction mixture was stirred at −10 °C for 3 h. The solid was filtered off and dried under vacuum. The product **66** (0.102 g, 0.5 mmol, 95%) was obtained as a colorless solid.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm) δ (ppm): 10.71 (bs, 3H, NH_3), 9.95 (bs, 1H, NH), 8.37 (bs, 1H, OH), 7.00 (dd, J = 2.0, 1.4 Hz, 1H, H-4), 6.95 (dd, J = 2.1, 1.4 Hz, 1H, H-2), 6.64 (t, J = 2.2 Hz, 1H, H-6); **^{13}C NMR** (100 MHz, DMSO-d_6, DMSO-d_6 = 39.5 ppm) δ (ppm): 167.2 (s, COOH), 158.1 (s, C-Ar), 147.4 (s, C-Ar), 132.4 (s, C-Ar), 109.2 (d, C-Ar), 105.9 (d, C-Ar), 105.6 (d, C-Ar); **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_7H_9N_2O_3]^+$: 169.0613, found: 169.0612.

3-Hydroxy-5-(hydroxymethyl)benzoic acid (48)



45 **46**

47 **48**

5-Hydroxyisophthalic acid (**45**) (10 g, 55 mmol, 1.0 eq.) was dissolved in MeOH (50 mL) and treated with conc. H_2SO_4 (0.5 mL). The reaction mixture was stirred under refluxing conditions for 20 h. After that time MeOH was removed under reduced pressure and the

residue was dissolved in EtOAc. The organic phase was washed with water (30 mL) and NaCl solution (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The product **46** was obtained as a colorless solid (11.2 g, 53.4 mmol, 97%) and used in the next step without further purification.

The analytical data are in accordance with the reported in the literature.[160]

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm) δ (ppm): 10.30 (s, 1H, Ar-OH), 7.92 (s, 1H, H-2), 7.55 (d, *J* = 1.5 Hz, 2H, H-4, H-6), 3.85 (s, 6H, COO*Me*); **^{13}C NMR** (100 MHz, DMSO-d_6, DMSO-d_6 = 39.5 ppm) δ (ppm): 165.4 (s, *C*OOMe), 157.9 (s, C-5), 131.4 (s, C-1, C-3), 120.3 (d, C-2), 120.1 (d, C-4, C-6), 52.4 (q, COO*Me*); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{10}H_{10}O_5Na]^+$: 233.0426, found: 233.0425; **M.p.**: 160–161 °C (Lit. 158–159 °C).[162]

Dimethyl 5-hydroxyisophthalate (**46**) (11.2 g, 53.4 mmol, 1.0 eq.) was dissolved in THF (150 mL), cooled down to 0 °C and treated with a $LiAlH_4$ solution (1 M in THF, 40 mmol, 40 mL, 0.75 eq.) which was added dropwise for 2 h. At the end of the addition 60 mL of THF was added to enable stirring. The suspension was stirred at RT for another 1.5 h. After that time the reaction was terminated by addition of water and THF was removed under reduced pressure. HCl (2 M, 30 mL) was added to the residue to destroy the aluminate complexes and $NaHCO_3$ was used to adjust pH of the solution to pH = 7. The obtained mixture was saturated with an aqueous NaCl solution and extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 3 : 1 to 1 : 1) to give the ester **47** (4.3 g, 23.6 mmol, 44%) as a colorless solid.

The analytical data are in accordance with the reported in the literature.[71]

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm) δ (ppm): 9.76 (s, 1H, Ar-OH), 7.38 (s, 1H, H-6), 7.21 (s, 1H, H-2), 6.99 (s, 1H, H-4), 5.27 (s, 1H, CH_2OH), 4.48 (s, 2H, CH_2OH), 3.82 (s, 3H, COO*Me*); **^{13}C NMR** (100 MHz, DMSO-d_6, DMSO-d_6 = 39.5 ppm) δ (ppm): 166.4 (s, *C*OOMe), 157.5 (s, C-3), 144.9 (s, C-5), 130.6 (s, C-1), 118.1 (d, C-Ar), 117.8 (d, C-Ar), 114.0 (d, C-Ar), 62.4 (t, CH_2OH), 52.1 (q, COO*Me*); **HRMS-ESI** (*m*/*z*): $[M-H]^-$ calcd for $[C_9H_9O_4]^-$: 181.0501, found: 181.0502; **M.p.**: 140–141 °C.

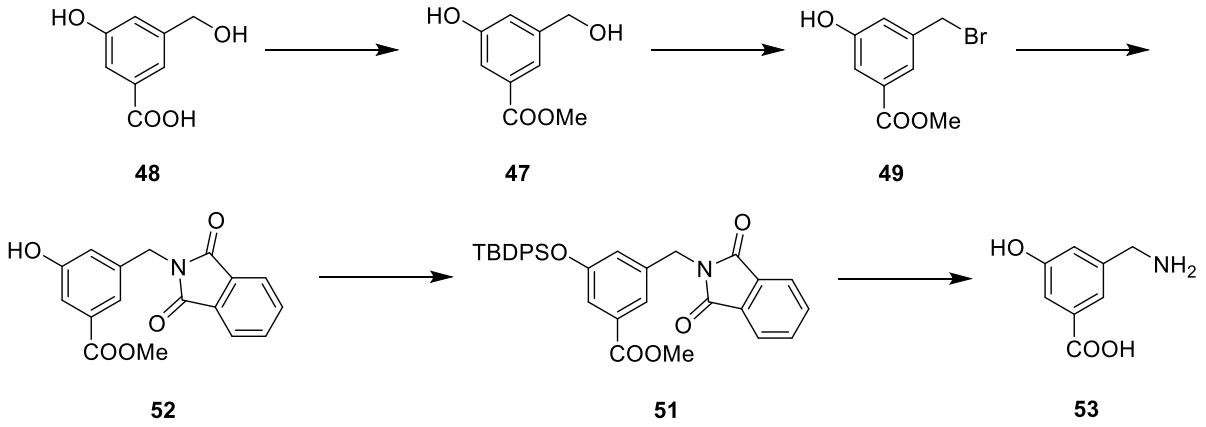
Methyl 3-hydroxy-5-(hydroxymethyl)benzoate (**47**) (2.5 g, 13.7 mmol, 1.0 eq.) was dissolved in MeOH (40 mL), treated with a 1 M solution of LiOH (68.6 mmol, 69 mL, 5.0 eq.) and the

mixture was stirred at RT for 2 h. After that time the solution was acidified to pH = 3 with 2 M HCl and extracted three times with EtOAc. The combined organic phased were dried over $MgSO_4$ and concentrated under reduced pressure to give the product **48** (2.2 g, 13.1 mmol, 96%) as a colorless solid.

The analytical data are in accordance with the reported in the literature.[70]

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm) δ (ppm): 9.68 (s, 1H, Ar-OH), 7.35 (s, 1H, H-6), 7.20 (s, 1H, H-2), 6.96 (s, 1H, H-4), 5.23 (s, 1H, $CH_2O\mathit{H}$), 4.46 (s, 2H, $C\mathit{H}_2OH$); **^{13}C NMR** (100 MHz, DMSO-d_6, DMSO-d_6 = 39.5 ppm) δ (ppm): 167.5 (s, *C*OOH), 157.3 (s, C-3), 144.6 (s, C-5), 131.8 (s, C-1), 118.1 (d, C-6), 117.7 (d, C-4), 114.2 (d, C-2), 62.5 (t, CH_2OH); **HRMS-ESI** (*m*/*z*): $[M-H]^-$ calcd for $[C_8H_7O_4]^-$: 167.0344, found: 167.0344; **M.p.**: 129–130 °C (Lit. 127 °C).[76]

3-(Aminomethyl)-5-hydroxybenzoic acid (53)



3-Hydroxy-5-(hydroxymethyl)benzoic acid (**48**) (561 mg, 3.34 mmol, 1.0 eq.) was dissolved in MeOH (90 mL) and treated with conc. H_2SO_4 (0.23 mL, 4.34 mmol, 1.3 eq.). The solution was heated under refluxing conditions for 24 h. After that time the solution of $NaHCO_3$ was added to the reaction mixture and MeOH was removed *in vacuo*. The aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The product **47** (608 mg, 3.34 mmol, quant.) was obtained as colorless crystals and used directly in the next step without further purification.

The analytical data are in accordance with the reported in the literature.[71]

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm) δ (ppm): 9.76 (s, 1H, Ar-OH), 7.38 (s, 1H, H-6), 7.21 (s, 1H, H-2), 6.99 (s, 1H, H-4), 5.27 (s, 1H, CH_2OH), 4.48 (s, 2H, CH_2OH), 3.82 (s, 3H, COO*Me*); **^{13}C NMR** (100 MHz, DMSO-d_6, DMSO-d_6 = 39.5 ppm) δ (ppm): 166.4 (s, *C*OOMe), 157.5 (s, C-3), 144.9 (s, C-5), 130.6 (s, C-1), 118.1 (d, C-Ar), 117.8 (d, C-Ar), 114.0 (d, C-Ar), 62.4 (t, CH_2OH), 52.1 (q, COO*Me*); **HRMS-ESI** (*m*/*z*): $[M-H]^-$ calcd for $[C_9H_9O_4]^-$: 181.0501, found: 181.0502.

Methyl 3-hydroxy-5-(hydroxymethyl)benzoate (**47**) (660 mg, 3.62 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (40 mL) and treated with PPh_3 (1.9 g, 7.25 mmol, 2.0 eq.) and CBr_4 (2.4 g, 7.25 mmol, 2.0 eq.). After the reaction was completed (3 h), silica gel was added to the reaction mixture, the solvent was removed *in vacuo* and the residue was applied on the column. Flash chromatography (PE : EA = 3 : 1 to 1 : 1) afforded the desired product **49** (795 mg, 3.25 mmol, 90%) as colorless crystals.

^{1}H NMR (400 MHz, acetone-d_6, acetone-d_5 = 2.05 ppm) δ (ppm): 8.89 (s, 1H, Ar-OH), 7.58 (t, *J* = 1.5 Hz, 1H, H-6), 7.42 (dd, *J* = 2.3, 1.4 Hz, H-2), 7.19 (t, *J* = 2.0 Hz, 1H, H-4), 4.64 (s, 2H, CH_2Br), 3.87 (s, 3H, COO*Me*); **^{13}C NMR** (100 MHz, acetone-d_6, acetone-d_6 = 206.26 ppm) δ (ppm): 166.7 (s, *C*OOMe), 158.5 (s, C-3), 141.2 (s, C-5), 132.9 (s, C-1), 122.1 (d, C-Ar), 121.4 (d, C-Ar), 116.8 (d, C-Ar), 52.4 (q, COO*Me*), 33.5 (t, CH_2Br).

Methyl 3-(bromomethyl)-5-hydroxybenzoate (**49**) (2.2 g, 8.98 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in DMF (120 mL) and treated with phthalimide potassium salt (6.65 g, 35.9 mmol, 4.0 eq.) and anhydrous K_2CO_3 (7.44 g, 53.9 mmol, 6.0 eq.). The reaction mixture was stirred at RT for 3 h after which the reaction was terminated by addition of an aqueous NH_4Cl solution. The mixture was extracted three times with Et_2O, the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (PE : EA = 4 : 1) providing the product **52** (1.7 g, 5.4 mmol, 60%) as a colorless solid.

^{1}H NMR (400 MHz, acetone-d_6, acetone-d_5 = 2.05 ppm) δ (ppm): 8.74 (s, 1H, ArOH), 7.90 – 7.84 (m, 4H, Ar), 7.53 (t, *J* = 1.9 Hz, 1H, H-2), 7.37 (t, *J* = 1.9 Hz, 1H, H-6), 7.11 (t, *J* = 1.9 Hz, 1H, H-4), 4.83 (s, 2H, ArCH_2), 3.84 (s, 3H, COO*Me*); **^{13}C NMR** (100 MHz, acetone-d_6, acetone-d_6 = 206.26 ppm) δ (ppm): 168.5 (s, Ar(CO)$_2$NH), 166.9 (s, *C*OOMe), 158.6 (s, C-5), 140.0 (s, C-3), 135.2 (d, Ar), 133.0 (s, C-1), 132.8 (s, Ar), 124.0 (d, Ar), 120.9 (d, C-2), 120.3 (d, C-4), 116.1 (d, C-6), 52.4 (q, COO*Me*), 41.6 (t, ArCH_2); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{17}H_{13}NO_5Na]^+$: 334.0691, found: 334.0691.

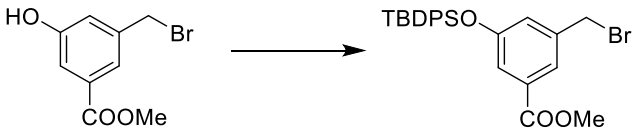
Methyl 3-[(1,3-dioxoisoindolin-2-yl)methyl]-5-hydroxybenzoate (**52**) (1.2 g, 3.85 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (60 mL) and treated successively with imidazole (0.53 g, 7.7 mmol, 2.0 eq.), 4-DMAP (47 mg, 0.39 mmol, 0.1 eq.) and TBDPSCl (1.3 mL, 5.0 mmol, 1.3 eq.). After 5 h the reaction was terminated by addition of an aqueous NH_4Cl solution and the phases were separated. The aqueous layer was extracted three times with CH_2Cl_2, the combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification by flash chromatography (PE : EA = 3 : 1 to 1 : 1) provided the product **51** (2.12 g, 3.85 mmol, quant.) as a colorless oil.

^{1}H NMR (400 MHz, acetone-d_6, acetone-d_5 = 2.05 ppm) δ (ppm): 7.90 – 7.83 (m, 4H, Ar), 7.70 – 7.64 (m, 4H, SiPh), 7.60 (t, *J* = 1.5 Hz, 1H, H-6), 7.40 – 7.29 (m, 6H, SiPh), 7.40 – 7.29 (m, 1H, H-2), 6.98 (dd, *J* = 2.4, 1.7 Hz, H-4), 4.72 (s, 2H, $ArCH_2$), 3.80 (s, 3H, COO*Me*), 1.10 (s, 9H, Si*t*-Bu); **^{13}C NMR** (100 MHz, acetone-d_6, acetone-d_6 = 206.26 ppm) δ (ppm): 168.2 (s, Ar(*C*O)$_2$NH), 166.5 (s, *C*OOMe), 156.8 (s, C-3), 139.8 (s, C-5), 136.2 (d, Ar), 135.1 (s, Ar), 132.9 (d, Ar), 132.8 (s, Ar), 132.6 (s, C-1), 131.1 (d, Ar), 128.7 (d, Ar), 124.2 (d, C-6), 124.0 (d, Ar), 122.6 (d, C-4), 120.5 (d, C-2), 52.4 (q, COO*Me*), 41.3 (t, Ar*C*H$_2$), 26.9 (q, Si*t*-Bu), 19.9 (s, Si*t*-Bu); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{33}H_{31}NO_5NaSi]^+$: 572.1869, found: 572.1869.

To a solution of phthalimide intermediate **51** (1.13 g, 2.05 mmol, 1.0 eq.) in EtOH (30 mL) hydrazine monohydrate (440 µL, 5.76 mmol, 2.8 eq.) was added. The reaction mixture was heated under refluxing conditions for 3 h, cooled down to RT and terminated by addition of water (20 mL). The reaction mixture was concentrated under reduced pressure to the final volume of ca 20 mL. Concentrated HCl (7 mL) was added and the reaction mixture was heated under refluxing conditions for another 2 h. After that time the mixture was cooled down to 0 °C and concentrated under reduced pressure to give the desired hydrochloride **53**·HCl (0.419 g, 2.05 mmol, quant.) as a colorless solid.

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm) δ (ppm): 10.16 (s, 1H, ArOH), 8.53 (bs, 2H, NH_2), 7.49 (t, *J* = 1.3 Hz, 1H, H-2), 7.37 (dd, *J* = 2.3, 1.5 Hz, 1H, H-6), 7.14 (t, *J* = 1.7 Hz, 1H, H-4), 3.97 (q, *J* = 5.6 Hz, 2H, $ArCH_2$); **^{13}C NMR** (100 MHz, DMSO-d_6, DMSO-d_6 = 39.5 ppm) δ (ppm): 167.1 (s, COOH), 157.8 (s, C-5), 135.8 (s, C-3), 132.3 (s, C-1), 120.6 (d, C-2), 120.6 (d, C-4), 116.0 (d, C-6), 41.9 (t, Ar*C*H$_2$); **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_8H_{10}NO_3]^+$: 168.0661, found: 168.0664; **M.p.**: 128–129 °C.

Methyl 3-(bromomethyl)-5-(*tert*-butyldiphenylsilyloxy)benzoate (50)

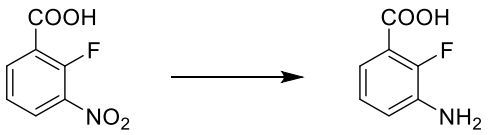


49 **50**

Methyl 3-(bromomethyl)-5-hydroxybenzoate (**49**) (0.84 g, 3.44 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (40 mL) and treated successively with imidazole (0.47 g, 6.88 mmol, 2.0 eq.), 4-DMAP (42 mg, 0.34 mmol, 0.1 eq.) and TBDPSCl (1.2 mL, 4.47 mmol, 1.3 eq.). After 5 h the reaction was terminated by addition of an aqueous NH_4Cl solution and the phases were separated. The aqueous layer was extracted three times with CH_2Cl_2, the combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification by flash chromatography (PE : EA = 10 : 1 to 4 : 1) provided the product **50** (1.61 g, 3.33 mmol, 97%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.72 – 7.70 (m, 4H, SiPh), 7.57 (s, 1H, H-2), 7.47 – 7.36 (m, 6H, SiPh), 7.47 – 7.36 (m, 1H, H-6), 6.91 – 6.89 (m, 1H, H-4), 4.37 (s, 2H, ArC*H*$_2$), 3.83 (s, 3H, COO*Me*), 1.14 (s, 9H, Si*t*-Bu); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.2 ppm) δ (ppm): 166.4 (s, *C*OOMe), 156.0 (s, C-5), 138.9 (s, C-3), 135.6 (d, C-Ar), 132.3 (s, C-Ar), 131.8 (s, C-1), 130.2 (d, C-Ar), 128.0 (d, C-Ar), 124.4 (d, C-2), 122.5 (d, C-4), 121.0 (d, C-6), 52.3 (q, COO*Me*), 45.4 (t, CH_2Br), 26.7 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu).

3-amino-2-fluorobenzoic acid (55)



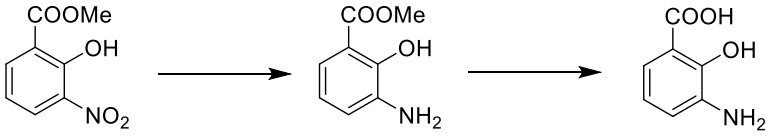
54 **55**

2-Fluoro-3-nitrobenzoic acid (**54**) (930 mg, 5 mmol, 1.0 eq.) was dissolved in EtOH (5 mL). Triethylsilane (8 mL, 50 mmol, 10.0 eq.) and 10% Pd/C (93 mg) were added and the reaction mixture was stirred at RT for 1 h. After that time the catalyst was filtered through a pad of Celite^{TM} and washed with EtOH. After removal of the solvent under reduced pressure, 3-amino-2-fluorobenzoic acid **55** was obtained as a pale yellow solid (756 mg, 4.9 mmol, 97%).

The analytical data are in accordance with the reported in the literature.[54,72]

^{1}H NMR (400 MHz, CD_3OD, CHD_2OD = 3.31 ppm) δ (ppm): 7.15 (ddd, J = 7.8, 6.4, 1.8 Hz, 1H, H-6), 7.01 (td, J = 7.9, 1.8 Hz, H-4), 6.98 – 6.89 (m, 1H, H-2); **^{1}H NMR** (400 MHz, DMSO-$_{d6}$, DMSO-$_{d5}$ = 2.50 ppm) δ (ppm): 7.03 – 6.85 (m, 3H), 5.30 (s, 2H, NH_2); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 168.2 (s, COOH), 152.0 (s, C-2), 138.2 (s, C-3), 124.8 (d, C-5), 121.9 (d, C-4), 120.5 (d, C-6), 120.2 (s, C-1); **HRMS-ESI** (*m/z*): $[M+H]^+$ calcd for $[C_7H_7FNO_2]^+$: 156.0461, found: 156.0457; **M.p.**: 180–181 °C.

3-amino-2-hydroxybenzoic acid (61)



59 **60** **61**

Methyl 2-hydroxy-3-nitrobenzoate (**59**) was prepared according to the literature procedure.[74]

Methyl 2-hydroxy-3-nitrobenzoate (**59**) (1 g, 5 mmol, 1.0 eq.) was dissolved in EtOH (5 mL) and 10% Pd/C (100 mg) was added. Afterwards triethylsilane (8.0 mL, 50 mmol, 10 eq.) was added dropwise to the reaction mixture under argon atmosphere. When the reaction was completed (monitored by TLC), the solution was filtered through a pad of Celite^{TM}. After removal of the solvent *in vacuo*, the product was purified by a flash column chromatography (PE : EA = 5 : 1). The solvents were removed under reduced pressure providing compound **60** (0.8 g, 4.8 mmol, 94%) as a yellow-brownish solid.

The analytical data are in accordance with the reported in the literature.[163]

^{1}H NMR (400 MHz, CD_3OD, CHD_2OD = 4.87 ppm) δ (ppm): 7.23 (dd, J = 8.0, 1.6 Hz, 1H, H-6), 6.96 (dd, J = 7.7, 1.6 Hz, 1H, H-4), 6.71 (t, J = 7.9 Hz, 1H, H-5), 3.95 (s, 3H, $COOCH_3$); **HRMS-ESI** (*m/z*): $[M+H]^+$ calcd for $[C_8H_{10}NO_3]^+$: 168.0661, found: 168.0666; **M.p.**: 86 °C (Lit. 88–89 °C).[163]

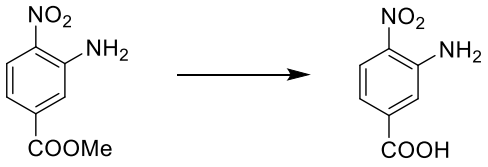
Methyl 3-amino-2-hydroxybenzoate (**60**) (0.3 g, 1.8 mmol, 1.0 eq.) was dissolved in MeOH (15 mL) and a 1 M solution of LiOH (18.0 mL, 10 eq.) was added dropwise. The solution was heated to 40 °C and stirred until complete conversion was observed (monitored by TLC). After cooling down to RT, the mixture was hydrolyzed by addition of crushed ice. The reaction mixture was acidified with 1 M HCl to pH = 2–3 and extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and after removal of the

solvent under reduced pressure the title compound **61** (0.24 g, 1.6 mmol, 88%) was obtained as dark brown crystals. No further purification was performed.

The analytical data are in accordance with the reported in the literature.[164,165]

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 7.37 (dd, J = 8.0, 1.6 Hz, 1H, H-6), 7.03 (dd, J = 7.7, 1.5 Hz, 1H, H-4), 6.70 (t, J = 7.9 Hz, 1H, H-5); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 174.1 (s, *C*OOH), 152.8 (s, C-2), 143.7 (s, C-3), 123.4 (s, C-6), 123.0 (d, C-4), 119.6 (d, C-5), 114.8 (d, C-1); **HRMS-ESI** (m/z): $[M+H]^+$ calcd for $[C_7H_8NO_3]^+$: 154.0504, found: 154.0502; **M.p.**: 232–233 °C (Lit. 231–235 °C).[164]

3-amino-4-nitrobenzoic acid (76)



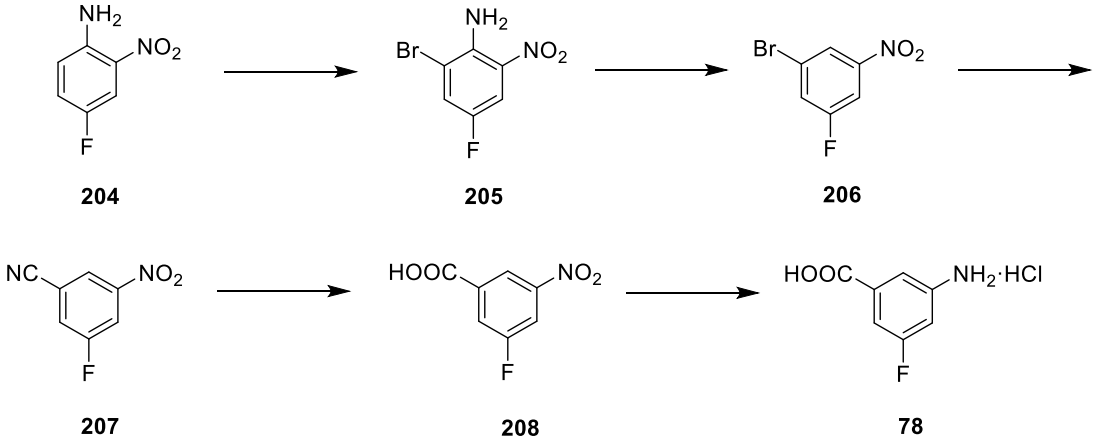
203 **76**

Methyl 3-amino-4-nitrobenzoate (**203**) was prepared according to the literature procedure.[166]

A solution of methyl 3-amino-4-nitrobenzoate (**203**) (500 mg, 2.55 mmol, 1.0 eq.) in MeOH (50 mL) and 1 M NaOH (13 mL) was stirred at RT for 1 h. After that time the reaction mixture was washed with Et_2O (20 mL). The pH of the aqueous phase was adjusted to 2 and the mixture was extracted three times with EtOAc. The combined organic extracts were collected and dried over $MgSO_4$. The solvent was removed under reduced pressure providing a crude product which was crystallized from EtOH to give 3-amino-4-nitrobenzoic acid **76** as yellow crystals (360 mg, 1.97 mmol, 78%).

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm) δ (ppm): 8.53 (d, J = 1.9 Hz, 1H, H-2), 7.92 (s, 2H, NH_2), 7.83 (dd, J = 8.9, 1.9 Hz, 1H, H-6), 7.04 (d, J = 8.9 Hz, 1H, H-5); **^{13}C NMR** (100 MHz, DMSO-d_6, DMSO-d_6 = 39.5 ppm) δ (ppm): 166.2 (s, COOH), 148.9 (s, C-3), 135.4 (s, C-4), 129.8 (s, C-1), 128.3 (s, C-5), 119.4 (s, C-2), 117.7 (s, C-6); **HRMS-ESI** (m/z): $[M-H]^-$ calcd for $[C_7H_5N_2O_4]^-$: 181.0249, found: 181.0255; **M.p.**: 295–297 °C.

3-Amino-5-fluorobenzoic acid hydrochloride (78·HCl)



3-Amino-5-fluorobenzoic acid hydrochloride (**78**·HCl) was prepared according to the literature procedure.[167]

4-Fluoro-2-nitroaniline (**204**) (5 g, 32 mmol, 1.0 eq.) was added to a solution of bromine (1.97 mL, 38 mmol, 1.2 eq.) in 20 mL of glacial acetic acid at 15 °C. The mixture was stirred at RT for 1 h, poured into 125 mL of water, stored overnight in the freezer and filtered. The residue was dissolved in MeOH, treated with charcoal and recrystallized from a MeOH : H_2O mixture providing 2-bromo-4-fluoro-6-nitroaniline (**205**) as an orange solid (5.4 g, 23 mmol, 72%).

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.90 (dd, J = 8.8, 3.0 Hz, 1H, H-5), 7.56 (dd, J = 7.0, 3.0 Hz, 1H, H-3), 6.49 (s, 2H, NH_2); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.2 ppm) δ (ppm): 152.1 (s, C-4), 139.6 (s, C-1), 139.6 (s, C-6), 127.8 (d, C-3), 112.3 (s, C-2), 111.8 (d, C-5); **M.p.**: 73–74 °C (Lit. 74–75 °C).[167]

2-Bromo-4-fluoro-6-nitroaniline (**205**) (1.5 g, 6.4 mmol, 1.0 eq.) was cooled down to −5 °C and conc. H_2SO_4 (15 mL) was added with vigorous stirring. Sodium nitrite (0.48 g, 7 mmol, 1.1 eq.) in 10 mL of water was added to the mixture at −2 to −3 °C over a period of 90 min (reaction progress monitored by TLC). After that time the mixture was stirred for further 15 min, cooled down to −5 °C and 50% H_3PO_2 (4.73 mL, 7.0 eq.) and Cu_2O (1 g, 7 mmol, 1.1 eq.) were added in small portions with vigorous stirring for the next 90 min. After that time the reaction mixture was poured into 250 g of crushed ice and stored in the freezer for 1 h. The precipitate was filtered and washed with ice-cold water. The aqueous layer was extracted with $CHCl_3$, the organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure to yield a dark oil. The crude product was purified by flash chromatography

(PE : EA = 5 : 1) to afford 3-bromo-5-fluoronitrobenzene (**206**) as a slightly yellow solid (0.84 g, 3.8 mmol, 60%).

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 8.20 (td, J = 1.8, 1.1 Hz, 1H, H-2), 7.89 (dt, J = 8.1, 2.2 Hz, 1H, H-6), 7.60 (ddd, J = 7.4, 2.4, 1.7 Hz, 1H, H-4); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.0 ppm) δ (ppm): 163.7 (s, C-5), 149.0 (s, C-1), 123.1 (s, C-3), 113.8 (d, C-2), 108.9 (d, C-4), 100.9 (d, C-6); **M.p.**: 27–28 °C (Lit. 27–28 °C).[167]

Copper (I) cyanide (550 mg, 6.1 mmol, 1.1 eq.) was stored *in vacuo* overnight. The flask was then purged with nitrogen and the mixture of 3-bromo-5-fluoronitrobenzene (**206**) (1.27 g, 5.75 mmol, 1.0 eq.) in DMF (10 mL) was added through a syringe. The reaction mixture was heated under reflux for 5 h. After that time it was diluted to 100 mL with 5 mL of 6 M HCl and 95 mL of water and extracted with EtOAc. The combined organic layers were washed with water and a solution of 5% $NaHCO_3$ and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (PE : EA = 4 : 1) providing 3-fluoro-5-nitrobenzonitrile **207** as a yellow solid (472 mg, 2,84 mmol, 49%).

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 8.36 (dt, J = 2.1, 1.2 Hz, 1H, H-6), 8.21 (dt, J = 7.8, 2.3 Hz, 1H, H-2), 7.73 (ddd, J = 7.1, 2.4, 1.3 Hz, 1H, H-4); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.0 ppm) δ (ppm): 162.3 (s, C-3), 149.6 (s, C-5), 125.2 (d, C-6), 123.3 (d, C-2), 116.2 (d, C-4), 115.58 (s, CN), 115.45 (s, C-1); **HRMS-EI** (*m*/*z*): $[M+H]^+$ calcd for $[C_7H_3FN_2O_2]^+$: 166.0179, found: 166.0181; **M.p.**: 49–51 °C (Lit. 49–50 °C).[167]

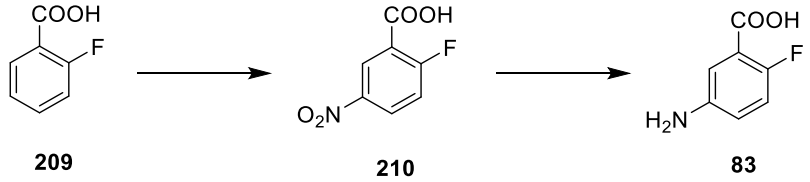
A mixture of 3-fluoro-5-nitrobenzonitrile (**207**) (470 mg, 2.83 mmol, 1.0 eq.) in 75% H_2SO_4 (6 mL) was stirred at 150 °C for 2 h. The reaction mixture was poured into ice-cold water and $NaHCO_3$ was added until gas evolution ceased. The solution was washed with Et_2O, then acidified to pH = 1 with 6 M HCl and extracted twice with Et_2O. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to give 3-fluro-5-nitrobenzoic acid (**208**) as a yellow solid (447 mg, 2.42 mmol, 85%).

^{1}H NMR (400 MHz, CD_3OD, CHD_2OD = 3.31 ppm, 4.87 ppm) δ (ppm): 8.51 (bs, 1H, H-6), 8.14 (dt, J = 8.2 Hz, 2.2 Hz, 1H, H-2), 8.00 (d, J = 8.3 Hz, 1H, H-4); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 166.1 (s, COOH), 163.8 (s, C-3), 150.8 (s, C-5), 136.2 (s, C-1), 123.4 (d, C-2), 121.4 (d, C-6), 115.95 (d, C-4); **HRMS-EI** (*m*/*z*): $[M-H]^-$ calcd for $[C_7H_3FNO_4]^-$: 184.0046, found: 184.0043, **M.p.**: 122–123 °C (Lit. 124–125 °C).[167]

3-Fluoro-5-nitrobenzoic acid (**208**) (300 mg, 1.62 mmol, 1.0 eq.) was dissolved in EtOH (20 mL) and treated with 6 M HCl (3 mL) and 10% Pd/C (45 mg). The mixture was stirred under hydrogen atmosphere for 3 h and then filtered through acid-washed Celite^{TM} pad. The solvent was removed under reduced pressure providing 3-amino-5-fluorobenzoic acid **78** as pale yellow crystals (242 mg, 1.3 mmol, 78%).

^{1}H NMR (400 MHz, CD_3OD, CHD_2OD = 3.31 ppm, 4.87 ppm) δ (ppm): 7.88 (dd, J = 2.3, 1.0 Hz, 1H, H-2), 7.82 (ddd, J = 8.8, 2.5, 1.3 Hz, 1H, H-6), 7.44 (dt, J = 8.6, 2.3 Hz, 1H, H-4); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 166.6 (s, COOH), 164.2 (s, C-5), 136.4 (s, C-3), 134.5 (s, C-1), 121.2 (d, C-2), 117.7 (d, C-6), 116.0 (d, C-4); **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_7H_7FNO_2]^+$: 156.0461, found: 156.0460; **M.p.**: 242–243 °C (Lit. 244 °C).[167]

5-Amino-2-fluorobenzoic acid (83)



209 **210** **83**

5-Amino-2-fluorobenzoic acid (**83**) was prepared according to the literature procedure.[168]

A mixture of fuming HNO_3 (2.8 mL) and conc. H_2SO_4 (8.2 mL) was added to a solution of 2-fluorobenzoic acid (**209**) (5 g, 36 mmol, 1.0 eq.) in conc. H_2SO_4 (19.6 mL). The reaction mixture was stirred at 90 °C for 45 min, then cooled down to RT, poured onto ice, filtered, washed with water and dried under vacuum providing 2-fluoro-5-nitrobenzoic acid (**210**) as colorless crystals (3.48 g, 18.8 mmol, 52%).

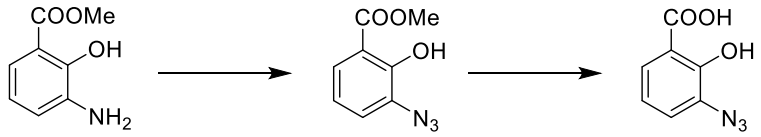
^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 8.96 (dd, J = 6.1, 2.9 Hz, 1H, H-6), 8.50 (ddd, J = 9.1, 3.9, 2.9 Hz, 1H, H-4), 7.39 (t, J = 9.3 Hz, 1H, H-3); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.2 ppm) δ (ppm): 167.2 (s, COOH), 165.8 (s, C-2), 144.1 (s, C-5), 130.8 (d, C-4), 129.0 (d, C-6), 118.8 (d, C-3), 118.9 (s, C-1); **HRMS-ESI** (*m*/*z*): $[M-H]^-$ calcd for $[C_7H_3FNO_4]^-$: 184.0046, found: 184.0048; **M.p.**: 140–141 °C (Lit. 141–142 °C).[168]

A mixture of 2-fluoro-5-nitrobenzoic acid (**210**) (3.4 g, 18.4 mmol, 1.0 eq.), 10% Pd/C (2.9 g), MeOH (40 mL) and cyclohexene (45 mL) was heated under refluxing conditions for 3 h. After that time the reaction mixture was filtered through a pad of Celite^{TM} to remove the

catalyst. The solvents were removed under reduced pressure to give a crude product which was crystallized from MeOH providing 5-amino-2-fluorobenzoic acid (**83**) as a colorless solid (2.13 g, 13.7 mmol, 75%).

^{1}H NMR (400 MHz, DMSO-$_{d6}$, DMSO-$_{d5}$ = 2.50 ppm) δ: 7.02 (dd, J = 6.2, 3 Hz, 1H, H-2), 6.93 (dd, J = 10.7, 8.8 Hz, 1H, H-5), 6.74 (ddd, J = 8.8, 4.1, 3.0 Hz, 1H, H-4), 3.17 (s, NH_2); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ(ppm): 167.8 (s, COOH), 157.0 (s, C-6), 143.5 (s, C-3), 122.7 (d, C-4), 120.3 (s, C-1), 119.2 (d, C-2), 118.2 (d, C-5); **HRMS-ESI** (m/z): $[M+H]^+$ calcd for $C_7H_6FNO_2$ 156.0461, found: 156.0461; $[M-H]^-$ calcd for $[C_7H_5FNO_2]^-$: 154.0304, found: 154.0307; **M.p.**: 190–191 °C (Lit. 190–192 °C).[168]

3-Azido-2-hydroxybenzoic acid (63)



60 **62** **63**

Methyl 3-amino-2-hydroxybenzoate (**60**) (0.74 g, 4.4 mmol, 1.0 eq.) was dissolved in 2 M HCl (20 mL) and cooled with an ice-salt mixture. Subsequently, an ice cold solution of sodium nitrite (0.37 g, 5.3 mmol, 1.2 eq.) in water (1.3 mL) was added dropwise and after 10 min of stirring urea (0.03 g, 0.52 mmol, 0.12 eq.) was added to destroy the excess of nitric acid. The formed diazonium salt solution was added dropwise to an ice cold solution of sodium azide (0.57 g, 8.8 mmol, 2.0 eq.) and sodium acetate (0.001 g, 0.01 mmol) in water (6.0 mL). After stirring for 2 h, the product was extracted three times with Et_2O and the combined organic phases were dried over Na_2SO_4. The solvent was removed under reduced pressure providing compound **62** (0.85 g, 4.4 mmol, quant.) as dark red crystals. The product was used directly in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 11.18 (s, 1H, OH), 7.62 (dd, J = 8.0, 1.6 Hz, 1H, H-6), 7.16 (dd, J = 7.9, 1.4 Hz, 1H, H-4), 6.85 (t, J = 7.9 Hz, 1H, H-5); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 170.5 (s, *C*$OOCH_3$), 154.6 (s, C-2), 128.3 (s, C-3), 126.1 (d, C-4), 126.0 (d, C-6), 119.3 (d, C-5), 113.5 (s, C-1), 52.8 (q, COO*C*H_3); **M.p.**: 72–73 °C.

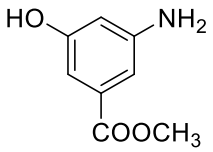
Methyl 3-azido-2-hydroxybenzoate (**62**) (0.53 g, 2.73 mmol, 1.0 eq.) was dissolved in MeOH (25 mL) and 1 M solution of LiOH (27.3 mL, 10.0 eq.) was added dropwise. The solution was

heated to 40 °C and stirred until complete conversion was observed (monitored by TLC). After cooling down to RT, the mixture was hydrolyzed by the addition of crushed ice. The reaction mixture was acidified with 1 M HCl to pH = 2–3 and extracted three times with EtOAc The combined organic phases were dried over $MgSO_4$ and after removal of the solvent under reduced pressure the title compound **63** (0.44 g, 2.5 mmol, 90%) was obtained as dark brown crystals. No further purification was conducted.

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 7.68 (dd, J = 8.0, 1.6 Hz, 1H, H-6), 7.17 (dd, J = 7.9, 1.6 Hz, 1H, H-4), 6.88 (t, J = 7.9 Hz, 1H, H-5); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 173.5 (s, *C*OOH), 156.4 (s, C-2), 129.0 (s, C-3), 127.9 (d, C-6), 127.1 (d, C-4), 120.1 (d, C-5), 115.1 (s, C-1); **HRMS-ESI** (*m*/*z*): $[M-H]^-$ calcd for $[C_7H_4N_3O_3]^-$: 178.0253, found: 178.0252; **M.p.**: 157–160 °C.

3.5 Synthesis of the Western Fragment

Methyl 3-amino-5-hydroxybenzoate (159)



$C_8H_9NO_3$
Mol. Wt.: 167.16 g/mol

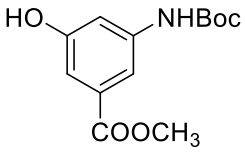
159

3,5-Dihydroxybenzoic acid (**65**) (22.5 g, 146 mmol, 1.0 eq.), ammonium chloride (19.1 g, 358 mmol, 2.45 eq.) and concentrated ammonia (30% in water, 67.5 mL) were placed in a steel bomb (model T304, PARR INSTRUMENT COMPANY) and heated at 180 °C for 40 h. After cooling down and evaporation of the reaction mixture to dryness, it was treated with MeOH (500 mL) and acetyl chloride (20.8 mL, 292 mmol, 2.0 eq.). The reaction mixture was heated under refluxing conditions for 36 h. After that time MeOH was removed under reduced pressure, the residue washed with ice-cold water and extracted three times with Et_2O (ether phases were discarded). The aqueous layer was neutralized with $NaHCO_3$ solution and extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product **159** was used in the following step without further purification.

The analytical data are in accordance with the reported in the literature.[141]

^{1}H NMR (400 MHz, acetone-d_6, acetone-d_5 = 2.05 ppm) δ (ppm): 8.40 (bs, 1H, OH), 6.93 (dd, J = 2.1, 1.5 Hz, 1H, H-4), 6.84 (dd, J = 2.3, 1.5 Hz, 1H, H-2), 6.48 – 6.50 (m, 1H, H-6), 4.78 (s, 2H, NH_2), 3.88 (s, 3H, OMe).

Methyl 3-(*tert*-butoxycarbonylamino)-5-hydroxybenzoate (159a)



$C_{13}H_{17}NO_5$
Mol. Wt.: 267.28 g/mol

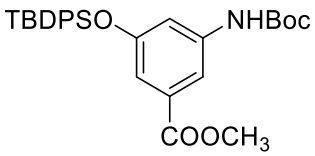
159a

Methyl ester **159** (17.7 g, 106 mmol, 1.0 eq.) was dissolved in a 1 : 4 (400 mL) mixture of THF : $NaHCO_3$ (400 mL) and treated with di-*tert*-butyl dicarbonate (23.1 g, 106 mmol, 1.0 eq.). After 1 h $NaHCO_3$ (10.7 g, 127 mmol, 1.2 eq.) was added and the mixture was stirred at RT for 44 h. THF was removed under reduced pressure and the remaining aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo* providing the crude solid product **159a** used in the following step without further purification.

The analytical data are in accordance with the reported in the literature.[141]

^{1}H NMR (400 MHz, acetone-d_6, acetone-d_5 = 2.05 ppm) δ (ppm): 8.59 (s, 1H, NH), 8.47 (bs, 1H, OH), 7.68 (s, 1H, H-4), 7.35 (s, 1H, H-2), 7.08 (s, 1H, H-6), 3.80 (s, 3H, OMe), 1.44 (s, 9H, *t*-Bu).

Methyl 3-(*tert*-butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)benzoate (160)



$C_{29}H_{35}NO_5Si$
Mol. Wt.: 505.69 g/mol

160

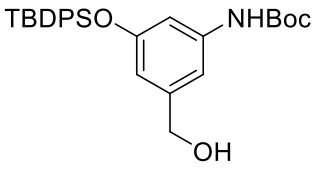
The ester **159a** (27.45 g, 102.7 mmol, 1.0 eq.) was dissolved in dry CH_2Cl_2 (257 mL) under argon atmosphere. The mixture was treated successively with imidazole (8.39 g, 123 mmol, 1.2 eq.), 4-DMAP (1.25 g, 10.3 mmol, 0.1 eq.) and TBDPSCl (28 mL, 108 mmol, 1.05 eq.) and stirred overnight at 30 °C. After the reaction was completed, an aqueous solution of

NH_4Cl was added and the phases were separated. The aqueous layer was extracted three times with CH_2Cl_2, the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash column chromatography (PE : EA = 20 : 1 to 10 : 1) afforded the desired product **160** as a colorless solid (43.4 g, 86 mmol, 84%).

The analytical data are in accordance with the reported in the literature.[141]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.72 – 7.69 (m, 4H, SiPh), 7.63 (s, 1H, ArH), 7.45 – 7.35 (m, 6H, SiPh), 7.09 (m, 1H, ArH), 6.96 (s, 1H, ArH), 6.37 (s, 1H, NH), 3.79 (s, 3H, OMe), 1.47 (s, 9H, *t*-Bu), 1.10 (s, 9H, Si*t*-Bu); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 166.7 (s, *C*OOMe), 156.2 (s, C-Ar), 152.4 (s, N*C*O*t*-Bu), 139.5 (s, C-Ar), 135.6 (d, C-Ar), 132.5 (s, C-Ar), 131.8 (s, C-Ar), 130.2 (d, C-Ar), 128.0 (d, C-Ar), 115.6 (d, C-Ar), 114.3 (d, C-Ar), 112.5 (d, C-Ar), 80.9 (s, *t*-Bu), 52.2 (q, OMe), 28.4 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{29}H_{35}NO_5NaSi]^+$: 528.2182, found: 528.2182.

3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)benzyl alcohol (161)



$C_{28}H_{35}NO_4Si$
Mol. Wt.: 477.68 g/mol

161

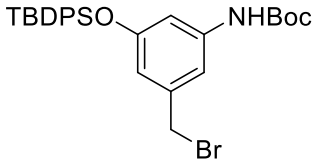
The ester **160** (20 g, 39.5 mmol, 1.0 eq.) was dissolved in dry THF (106 mL) under argon atmosphere and cooled down to −78 °C. After addition of DIBAL-H (119 mL, 1 M solution in hexane, 3.0 eq.) the reaction mixture was warmed up to 0 °C and stirred overnight at RT. The reaction was terminated by careful addition of saturated K-Na-tartrate solution. After extraction with Et_2O (4x) the combined organic layers were washed with NaCl solution (1x), dried over $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (PE : EA = 5: 1 to 1 : 1) affording a colorless foamy solid **161** (17.7 g, 37.1 mmol, 94%).

The analytical data are in accordance with the reported in the literature.[141]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.73 – 7.68 (m, 4H, SiPh), 7.45 – 7.34 (m, 6H, SiPh), 7.08 (s, 1H, ArH), 6.58 (s, 1H, AHr), 6.40 (s, 1H, ArH), 6.29 (bs, 1H,

NH), 4.42 (s, 2H, C*H*$_2$OH), 1.61 (bs, 1H, OH), 1.47 (s, 9H, *t*-Bu), 1.08 (s, 9H, Si*t*-Bu); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 156.4 (s, C-Ar), 152.6 (s, NCO*t*-Bu), 143.1 (s, C-Ar), 139.5 (s, C-Ar), 135.6 (d, C-Ar), 132.9 (s, C-Ar), 130.1 (d, C-Ar), 127.9 (d, C-Ar), 113.0 (d, C-Ar), 109.8 (d, C-Ar), 109.1 (d, C-Ar), 80.7 (s, *t*-Bu), 65.2 (t, CH_2), 28.4 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu); HRMS-ESI (m/z): [M+Na]+ calcd for $[C_{28}H_{35}NO_4NaSi]^+$: 500.2233, found: 500.2236.

3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)benzyl bromide (156)



$C_{28}H_{34}BrNO_3Si$
Mol. Wt.: 540.5730 g/mol

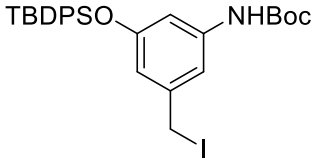
156

To a solution of the alcohol **161** (5.62 g, 11.8 mmol, 1.0 eq.) in dry CH_2Cl_2 (100 mL) PPh_3 (3.725 g, 14.2 mmol, 1.2 eq.) was added, followed by CBr_4 (4.7 g, 14.2 mmol, 1.2 eq.). After the reaction was completed (ca. 30 min), the solvent was removed under reduced pressure and the residue was applied to silica gel. The flash column chromatography (PE : EA = 10 : 1 to 5 : 1) afforded the desired product **156** as a colorless oil (6.31 g, 11.7 mmol, 99%).

The analytical data are in accordance with the reported in the literature.[141]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.73 – 7.67 (m, 4H, SiPh), 7.44 – 7.37 (m, 6H, SiPh), 7.16 (s, 1H, ArH), 6.55 (s, 1H, ArH), 6.43 (s, 1H, ArH), 6.25 (s, 1H, NH), 4.22 (s, 2H, C*H*$_2$Br), 1.47 (s, 9H, *t*-Bu), 1.08 (s, 9H, Si*t*-Bu); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 156.3 (s, C-Ar), 152.5 (s, N*C*O*t*-Bu), 139.5 (s, C-Ar), 135.6 (s, C-Ar), 132.7 (s, C-Ar), 130.1 (d, C-Ar), 128.0 (d, C-Ar), 115.3 (d, C-Ar), 111.9 (d, C-Ar), 109.9 (d, C-Ar), 80.8 (s, *t*-Bu), 33.45 (t, CH_2Br), 28.4 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{28}H_{34}NO_3NaSiBr]^+$: 562.1389, found: 562.1391.

3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)benzyl iodide (162)



$C_{28}H_{34}INO_3Si$
Mol. Wt.: 587.57 g/mol

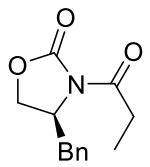
162

The bromide **156** (6.43 g, 11.9 mmol, 1.0 eq.) was dissolved in dry acetone (51 mL) and sodium iodide (2.68 g, 17.8 mmol, 1.5 eq.) was added. The reaction mixture was stirred at RT for 2 h. After the reaction was completed, distilled water was added and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with NaCl solution and dried over $MgSO_4$. The solvent was removed under reduced pressure and the product **162** was obtained as a brownish foam (6.64 g, 11.3 mmol, 95%).

The analytical data are in accordance with the reported in the literature.[62,141]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.73 – 7.68 (m, 4H, SiPh), 7.45 – 7.36 (m, 6H, SiPh), 7.12 (s, 1H, ArH), 6.55 – 6.54 (m, 1H, ArH), 6.41 – 6.39 (m, 1H, ArH), 6.27 (s, 1H, NH), 4.18 (s, 2H, C*H*$_2$I), 1.48 (s, 9H, *t*-Bu), 1.09 (s, 9H, Si*t*-Bu); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 156.2 (s, N*C*O*t*-Bu), 140.9 (s, C-Ar), 139.5 (s, C-Ar), 135.6 (d, C-Ar), 132.7 (s, C-Ar), 130.1 (d, C-Ar), 128.0 (d, C-Ar), 115.1 (d, C-Ar), 111.6 (d, C-Ar), 109.6 (d, C-Ar), 80.8 (s, *t*-Bu), 28.4 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu), 5.6 (t, CH_2I); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{28}H_{34}NO_3NaSiI]^+$: 610.1250, found: 610.1251.

(4*S*)-4-Benzyl-3-propionyl-2-oxazolidinone (166)



$C_{13}H_{15}NO_3$
Mol. Wt.: 233.27 g/mol

166

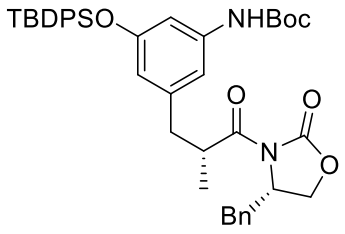
To a cold (−78 °C) solution of (4*S*)-4-benzyl-2-oxazolidinone (**166a**) (2.19 g, 12.4 mmol) in THF (40 mL) *n*-BuLi (2.5 M solution in hexanes) (5.2 mL, 13.0 mmol) was added dropwise over a period of 10 min. The reaction mixture was stirred at −78 °C for 10 min and afterwards

propionyl chloride (1.18 mL, 1.26 g, 13.6 mmol) was added. The mixture was stirred at −78 °C for 1 h and then slowly warmed up to RT over 30 min. The reaction was terminated by the addition of saturated NH_4Cl solution (8 mL). THF was removed under reduced pressure and the residue was extracted three times with CH_2Cl_2. The combined organic layers were washed with 1 M NaOH (20 mL) and NaCl solution (20 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (PE : EA = 4 : 1) to yield the desired compound **166** as a colorless crystalline solid (2.47 g, 10.6 mmol, 88%).

The analytical data are in accordance with the reported in the literature.[169,170]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.31 – 7.11 (m, 5H, ArH), 4.62 − 4.56 (m, 1H, NCH), 4.16 – 4.03 (m, 2H, CHCH_2O), 3.22 (dd, J = 13.4, 3.2 Hz, 1H, CH_2C$_6$H$_5$), 2.97 – 2.77 (m, 2H, CH_2CH$_3$), 2.69 (dd, J = 13.4, 9.6 Hz, 1H, CH_2C$_6$H$_5$), 1.12 (t, J = 7.4 Hz, 3H, CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 174.2 (s, *C*OCH$_2$CH$_3$), 153.6 (s, NCOO), 135.4 (d, C-Ar), 129.5 (d, C-Ar), 129.1 (d, C-Ar), 127.4 (d, C-Ar), 66.3 (t, CHCH$_2$O), 55.3 (q, NCH), 38.0 (s, N*C*OCH$_2$), 29.3 (t, *C*H$_2$CH$_3$), 8.4 (d, CH$_2$*C*H$_3$); **[α]$^{20}_D$** = 99.0° (*c* = 1.0, EtOH; Lit. [α]$^{20}_D$ = 99.5°, *c* = 1.01, EtOH)[169]; **M.p.**: 43–44 °C (Lit. 43–46 °C).[171]

(2*R*,4*S*)-3-{3-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)-phenyl]-2-methylpropanoyl}-4-benzyloxazolidin-2-one (163)



$C_{41}H_{48}N_2O_6Si$
Mol. Wt.: 692.93 g/mol

163

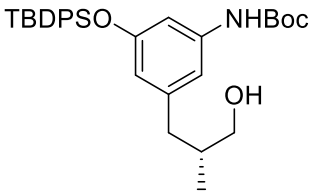
Diisopropylamine (4.30 mL, 30.8 mmol, 1.7 eq.) was dissolved in dry THF (150 mL) under argon atmosphere and cooled down to −78 °C. After the addition of *n*-BuLi (2.5 M solution in hexanes, 12.3 mL, 30.8 mmol, 1.7 eq.) the solution was stirred at 0 °C for 15 min and agaib cooled down to −78 °C. The oxazolidinone **166** (6.90 g, 29.6 mmol, 1.63 eq.) was dissolved in THF (100 mL) and cooled down to −78 °C. The prepared solution of LDA was added dropwise and the reaction mixture was warmed up to −40 °C. After cooling down to −78 °C

the iodide **163** (10.7 g, 18.1 mmol, 1.0 eq.) dissolved in THF (100 mL) was added to the reaction mixture. The mixture was slowly (ca 2 h) warmed up to −35 °C and stirred at this temperature for 13 h. The mixture was then hydrolyzed with a cold saturated solution of NH_4Cl and warmed up to RT. THF was removed under reduced pressure and the remaining aqueous phase was extracted with EtOAc (3x). After drying over $MgSO_4$ and concentrating of the solvent *in vacuo*, the residue was purified by column chromatography (PE : EA = 10 : 1 to 5 : 1). The pure diastereomer **163** (7.52 g, 10.8 mmol, 60%) was obtained as a colorless foam.

The analytical data are in accordance with the reported in the literature.[57,62]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.75 – 7.66 (m, 4H, SiPh), 7.39 – 7.27 (m, 6H, SiPh), 7.12 – 7.09 (m, 2H, ArH), 6.86 (bs, 1H, ArH), 6.73 (bs, 1H, ArH), 6.29 – 6.28 (m, 1H, ArH), 6.27 (bs, 1H, NH), 4.68 – 4.59 (m, 1H, CHBn), 4.19 – 4.09 (m, 2H, C*H*$_2$CHBn), 3.86 – 3.81 (m, 1H, C*H*CH_3), 3.16 (dd, *J* = 13.4, 3.2 Hz, 1H, C*H*$_2$Ph), 2.93 (dd, *J* = 13.2, 6.2 Hz, 1H, C*H*$_2$CHCH$_3$), 2.61 (dd, *J* = 13.2, 8.4 Hz, 1H, C*H*$_2$Ph), 2.33 (dd, *J* = 13.2, 8.4 Hz, 1H, C*H*$_2$CHCH$_3$), 1.45 (s, 9H, *t*-Bu), 1.07 (s, 9H, Si*t*-Bu), 0.93 (d, *J* = 6.7 Hz, 3H, CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 176.6 (s, N*C*OCH), 156.0 (s, C-Ar), 153.1 (s, NCOO*t*-Bu), 152.5 (s, NCOO), 140.9 (s, C-Ar), 139.3 (s, C-Ar), 135.64 (d, C-Ar), 135.62 (d, C-Ar), 132.97 (s, C-Ar), 132.93 (s, C-Ar), 130.0 (d, C-Ar), 129.5 (d, C-Ar), 129.0 (d, C-Ar), 127.9 (d, C-Ar), 127.4 (d, C-Ar), 115.8 (d, C-Ar), 112.1 (d, C-Ar), 108.2 (d, C-Ar), 80.4 (s, *t*-Bu), 66.1 (t, CH_2O), 55.3 (d, CHN), 39.6 (t, *C*H_2Ph), 39.5 (d, *C*HCH_3) 38.0 (d, *C*H_2CH), 28.4 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu), 16.2 (q, CH_3); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{41}H_{48}N_2O_6NaSi]^+$: 715.3179, found: 715.3177; **$[\alpha]^{20}_D$** = −3.4° (*c* = 1.5, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −3.4°, *c* = 1.5, $CHCl_3$).[62]

(2*R*)-3-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)-phenyl]-2-methylpropan-1-ol (163a)



$C_{31}H_{41}NO_4Si$
Mol. Wt.: 519.76 g/mol

163a

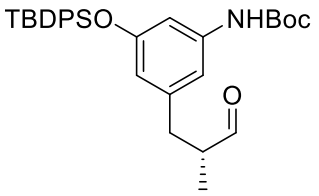
The oxazolidinone **163** (10.2 g, 14.7 mmol, 1.0 eq.) was dissolved in Et_2O (70 mL) under argon atmosphere, water (0.23 mL, 14.7 mmol, 1.0 eq.) was added and the mixture was

cooled down to 0 °C. Then $LiBH_4$ (2 M solution in THF) (16.2 ml, 32.4 mmol, 2.2 eq.) was added dropwise and the reaction mixture was stirred at 0 °C for 1.5 h. The reaction was terminated by addition of an aqueous NH_4Cl solution and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (PE : EA = 10 : 1 to 2 : 1) providing the alcohol **163a** (5.95 g, 11.5 mmol, 78%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[62]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.74 – 7.70 (m, 4H, SiPh), 7.44 – 7.34 (m, 6H, SiPh), 6.81 (bs, 1H, ArH), 6.69 (t, *J* = 2.0 Hz, 1H, Ph), 6.33 (bs, 1H, NH), 6.11 (t, *J* = 1.7 Hz, 1H, Ph), 3.24 (d, *J* = 3.8 Hz, 1H, C*H*$_2$OH), 3.20 (d, *J* = 3.8 Hz, 1H, C*H*$_2$OH), 2.41 (dd, *J* = 13.3, 6.8 Hz, 1H, ArC*H*$_2$), 2.16 (dd, *J* = 13.3, 6.8 Hz, 1H, ArC*H*$_2$), 1.70 – 1.60 (m, 1H, C*H*CH$_3$), 1.49 (s, 9H, *t*-Bu), 1.24 (bs, 1H, OH), 1.08 (s, 9H, Si*t*-Bu), 0.70 (d, *J* = 6.8 Hz, 3H, CHC*H*$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 156.00 (s, C-Ar), 152.7 (s, NCOO), 142.5 (s, C-Ar), 139.2 (s, C-Ar), 135.6 (d, C-Ar), 133.0 (s, C-Ar), 130.0 (d, C-Ar), 127.9 (d, C-Ar), 115.5 (d, C-Ar), 112.1 (d, C-Ar), 107.7 (d, C-Ar), 80.5 (s, *t*-Bu), 67.3 (t, CH_2), 39.6 (t, CH_2), 37.5 (d, CH), 28.5 (q, s, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.5 (s, Si*t*-Bu), 16.5 (q, CH_3); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{31}H_{41}NO_4NaSi]^+$: 542.2703, found: 542.2700; **$[\alpha]^{20}_D$** = +4.0° (*c* = 1.2, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = +4.9°, *c* = 1.0, $CHCl_3$).[62]

(2*R*)-3-[3-(tert-Butoxycarbonylamino)-5-(tert-butyldiphenylsiloxy)phenyl]-2-methylpropanal (155)



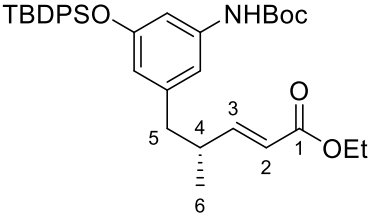
$C_{31}H_{39}NO_4Si$
Mol. Wt.: 517.74 g/mol

155

The alcohol **163a** (5.90 g, 11.4 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (170 mL), cooled down to 0 °C and treated successively with $NaHCO_3$ (1.16 g, 13.6 mmol, 1.2 eq.) and DMP reagent (5.76 g, 13.6 mmol, 1.2 eq.). After 1 h the reaction was terminated by addition of an aqueous Na_2SO_3 solution and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with the aqueous solution of NaCl, dried over $MgSO_4$

and concentrated under reduced pressure. The aldehyde **155** (5.80 g, 11.2 mmol, 99%) was obtained as a colorless foam. The product was used directly in the next step without further characterization.

Ethyl (4*R*)-5-[3-(*tert*-butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)phenyl]-4-methylpent-2-enoate (**164**)



$C_{35}H_{45}NO_5Si$
Mol. Wt.: 587.83 g/mol

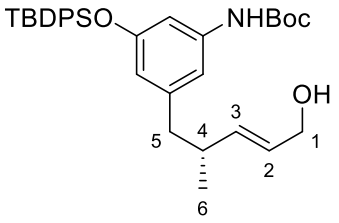
164

The aldehyde **155** (5.80 g, 11.2 mmol, 1.0 eq.) was dissolved in $CHCl_3$ (35 mL) and the phosphorus ylide **167** (5.85 g, 16.8 mmol, 1.5 eq.) was added. The reaction mixture was heated to 50 °C and stirred at 50 °C for additional 15 h. The solvent was removed under reduced pressure, the crude product was purified by flash chromatography (PE : EA = 15 : 1 to 4 : 1) and the ester **164** (4.7 g, 8.0 mmol, 72%) was obtained as a colorless foam.

The analytical data are in accordance with the reported in the literature.[136]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.72 – 7.67 (m, 4H, SiPh), 7.45 – 7.33 (m, 6H, SiPh), 6.81 (dd, *J* = 15.2, 6.9 Hz, 1H, 3-H), 6.79 (s, 1H, Ph), 6.64 (s, 1H, Ph), 6.24 (s, 1H, NH), 6.09 (s, 1H, Ph), 5.63 (dd, *J* = 15.9, 0.6 Hz, 1H, 2-H), 4.16 (q, *J* = 7.2 Hz, 2H, OC*H*$_2$), 2.54 – 2.47 (m, 1H, 5-Ha), 2.27 (dq, *J* = 6.8, 6.7 Hz, 1H, 4-H), 2.24 – 2.10 (m, 1H, 5-Hb), 1.48 (s, 9H, *t*-Bu), 1.27 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3), 1.08 (s, 9H, Si*t*-Bu), 0.78 (d, *J* = 6.1 Hz, 3H, 6-H); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 166.9 (s, COOEt), 156.1 (s, C-Ar), 153.7 (s, C-3), 152.6 (s, NHCOO), 141.5 (s, C-Ar), 139.2 (s, C-Ar), 133.0 (d, C-Ar), 133.0 (s, C-Ar), 130.0 (d, C-Ar), 130.0 (d, C-Ar), 127.9 (d, C-Ar), 119.8 (d, C-2), 115.3 (d, C-Ar), 120.0 (d, C-Ar), 107.9 (d, C-Ar), 80.5 (s, *t*-Bu), 60.3 (t, OCH_2), 42.2 (t, C-5), 37.9 (d, C-4), 28.5 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu), 18.5 (q, C-6), 14.4 (q, OCH_2CH_3); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{35}H_{45}NO_5NaSi]^+$: 610.2965, found: 610.2969; $\mathbf{[\alpha]^{20}_D}$ = −19.0° (*c* = 1.3, CH_2Cl_2; Lit. $[\alpha]^{20}_D$ = −18.9°, *c* = 1.3, CH_2Cl_2).[136]

(4*R*)-5-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)phenyl]-4-methylpent-2-enol (154)



$C_{33}H_{43}NO_4Si$
Mol. Wt.: 545.79 g/mol

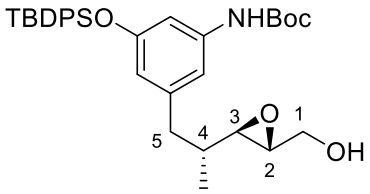
154

The ester **164** (5.23 g, 8.9 mmol, 1.0 eq) was dissolved in CH_2Cl_2 (85 mL) under argon atmosphere, cooled down to −78 °C and DIBAL-H (1.0 M solution in toluene) (22.3 mL, 22.3 mmol, 2.5 eq) was slowly added. The reaction mixture was stirred at −78 °C for 5 h and then warmed up to RT. The reaction was terminated by addition of an aqueous Na-K tartrate solution, and the reaction mixture was stirred at RT overnight. The aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and the solvent was concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 10 : 1 to 2 : 1) providing the alcohol **154** (3.65 g, 6.7 mmol, 75%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[136]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.72– 7.70 (m, 4H, SiPh), 7.44 – 7.34 (m, 6H, SiPh), 6.92 (s, 1H, Ph), 6.48 (m, 1H, Ph), 6.28 (bs, 1H, NH), 6.15 (m, 1H, Ph), 5.50 (dd, *J* = 15.5, 7.0 Hz, 1H, 3-H), 5.39 (dt, *J* = 15.5, 5.5 Hz, 1H, 2-H), 3.99 (t, *J* = 5.5 Hz, 2H, 1-H), 2.38 (dd, *J* = 13.1, 7.3 Hz, 1H, 5-Ha), 2.28 (dd, *J* = 13.1, 6.7 Hz, 1H, 5-Hb), 2.16 (m, 1H, 4-H), 1.79 (bs, 1H, $CH_2O\mathit{H}$), 1.48 (s, 9H, *t*-Bu), 1.08 (s, 9H, Si*t*-Bu), 0.80 (d, *J* = 6.8 Hz, 3H, 6-H); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 155.8 (s, C-Ar), 152.9 (s, NCOO), 142.4 (s, C-Ar), 138.7 (s, C-Ar), 138.0 (d, C-3), 135.6 (d, C-Ar), 133.0 (s, C-Ar), 130.0 (d, C-Ar), 127.9 (d, C-2), 115.7 (d, C-Ar), 112.9 (d, C-Ar), 107.7 (d, C-Ar), 80.6 (s, *t*-Bu), 63.9 (t, C-1), 43.4 (t, C-5), 37.8 (d, C-4), 28.5 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.7 (q, 6-Me), 19.6 (s, Si*t*-Bu); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{33}H_{43}NO_4NaSi]^+$: 568.2859, found: 568.2858; $\mathbf{[\alpha]^{20}_D}$ = −10.0° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −1.1°, *c* = 1.0, CH_2Cl_2).[136]

(2*R*,3*R*,4*R*)-5-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)phenyl]-4-methylpent-2,3-oxiranylpentan-2-ol (165)



$C_{33}H_{43}NO_5Si$
Mol. Wt.: 561.79 g/mol

165

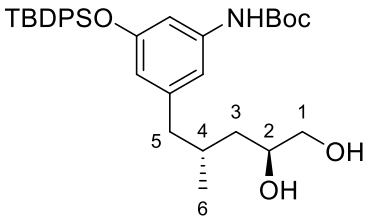
Molecular sieves 4Å (20.0 g) were suspended in CH_2Cl_2 under argon atmosphere (400 mL), cooled down to −20 °C and D-(−)-diethyl tartrate (1.75 mL, 10.0 mmol, 1.2 eq) and titanium isopropoxide (2.50 mL, 8.3 mmol, 1.0 eq) were added. The reaction mixture was stirred at −20 °C for 30 min and then treated with *tert*-butylhydroperoxide (2.00 mL, 10.8 mmol, 1.3 eq.). After 30 min, the alcohol **154** (4.54 g, 8.3 mmol, 1.0 eq) in CH_2Cl_2 (50 mL) was added to the reaction mixture and the reaction mixture was stirred at −20 °C for 20 h. The reaction was terminated by addition of an aqueous NaOH solution (2.0 M). After stirring for 3 h the reaction mixture was filtered through Celite™ and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 10 : 1 to 2 : 1) yielding the epoxide **165** (3.7 g, 6.6 mmol, 80%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[136]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.72 – 7.69 (m, 4H, SiPh), 7.43 – 7.34 (m, 6H, SiPh), 6.84 (s, 1H, Ph), 6.64 (m, 1H, Ph), 6.34 (bs, 1H, NH), 6.12 (m, 1H, Ph), 3.81 (ddd, *J* = 12.5, 5.9, 2.5 Hz, 1H, 1-Ha), 3.56 (ddd, *J* = 12.5, 6.9, 3.9 Hz, 1H, 1-Hb), 2.84 (ddd, *J* = 4.3, 2.6, 2.4 Hz, 1H, 2-H), 2.70 (dd, *J* = 6.9, 2.3 Hz, 1H, 3-H), 2.61 (dd, *J* = 13.3, 5.1 Hz, 1H, 5-Ha), 2.22 (dd, *J* = 13.2, 8.8 Hz, 1H, 5-Hb), 1.91 (bs, 1H, OH), 1.54 – 1.50 (m, 1H, 4-H), 1.48 (s, 9H, *t*-Bu), 1.08 (s, 9H, Si*t*-Bu), 0.59 (d, *J* = 6.8 Hz, 3H, 5-Me); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 156.00 (s, C-Ar), 152.7 (s, NCOO), 141.4 (s, C-Ar), 139.1 (s, C-Ar), 135.6 (d, C-Ar), 135.6 (d, C-Ar), 133.0 (s, C-Ar), 132.9 (s, C-Ar), 130.0 (d, C-Ar), 130.0 (d, C-Ar), 127.9 (d, C-Ar),), 127.9 (d, C-Ar), 115.6 (d, C-Ar), 112.4 (d, C-Ar), 107.9 (d, C-Ar), 80.6 (s, *t*-Bu), 61.8 (t, C-1), 59.9 (d, C-3), 57.2 (d, C-2), 40.1 (t, C-5), 36.4 (d, C-4), 28.5 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu), 14.8 (q, 5-Me);

HRMS-ESI (*m*/*z*): $[M+Na]^+$ calcd for $[C_{33}H_{43}NO_5NaSi]^+$: 584.2808, found: 584.2809; $[\alpha]^{20}_D$ = +9.2° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = +2.2°, *c* = 0.9, CH_2Cl_2).[136]

(2*S*,4*R*)-5-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)phenyl]-4-methyl-1,2-pentandiol (168)



$C_{33}H_{45}NO_5Si$
Mol. Wt.: 563.81 g/mol

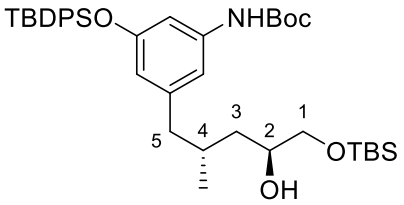
168

The epoxide **165** (3.45 g, 6.1 mmol, 1.0 eq.) was dissolved in Et_2O (220 mL) under argon atmosphere, cooled down to 0 °C and treated with DIBAL-H (1.0 M solution in hexanes) (31 mL, 31.0 mmol, 5.0 eq.). The reaction mixture was warmed up to RT and stirred for additional 5 h. Then it was washed with Na-K tartrate solution and stirred overnight. The aqueous phase was extracted three times with EtOAc, the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 1 : 1 to 1 : 4) and the diol **168** (3.0 g, 5.2 mmol, 85%) was obtained as a colorless foam.

The analytical data are in accordance with the reported in the literature.[136]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.73 – 7.68 (m, 4H, SiPh), 7.41 – 7.34 (m, 6H, SiPh), 6.80 (bs, 1H, Ph), 6.63 (m, 1H, Ph), 6.26 (bs, 1H, NH), 6.11 (m, 1H, Ph), 3.72 – 3.65 (m, 1H, 2-H), 3.52 (ddd, *J* = 10.8, 5.8, 3.3 Hz, 1H, 1-Ha), 3.30 (ddd, *J* = 10.8, 7.3, 4.0 Hz, 1H, 1-Hb), 2.31 (dd, *J* = 13.3, 6.6 Hz, 1H, 5-Ha), 2.19 (dd, *J* = 13.3, 7.8 Hz, 1H, 5-Hb), 1.86 (dd, *J* = 5.8, 4.0 Hz, 1H, 1-OH), 1.77 (d, *J* = 4.4 Hz, 1H, 2-OH), 1.73 – 1.64 (m, 1H, 4-H), 1.47 (s, 9H, *t*-Bu), 1.31 (ddd, *J* = 13.8, 9.3, 4.6 Hz, 1H, 3-Ha), 1.07 (s, 9H, Si*t*-Bu), 0.97 (ddd, *J* = 13.8, 9.7 3.9 Hz, 1H, 3-Hb); 0.69 (d, *J* = 6.4 Hz, 3H, 6-H); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 155.9 (s, C-Ar), 152.7 (s, NCOO), 142.6 (s, C-Ar), 139.0 (s, C-Ar), 135.6 (d, C-Ar), 133.0 (s, C-Ar), 129.9 (d, C-Ar), 127.8 (d, C-Ar), 115.5 (d, C-Ar), 112.3 (d, C-Ar), 107.9 (d, C-Ar), 80.5 (s, *t*-Bu), 70.1 (d, C-2), 67.5 (t, C-1), 44.0 (t, C-5), 39.7 (t, C-3), 30.8 (d, C-4), 28.4 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.5 (s, Si*t*-Bu), 14.7 (q, 6-Me); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{33}H_{45}NO_5NaSi]^+$: 586.2965, found: 586.2969; $[\alpha]^{20}_D$ = −10.1° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −11.0°, *c* = 0.6, CH_2Cl_2).[136]

(2*S*,4*R*)-5-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)-phenyl]-4-methyl-1-(*tert*-butyldemethylsiloxy)-pentan-2-ol (169)



$C_{39}H_{59}NO_5Si_2$
Mol. Wt.: 678.07 g/mol

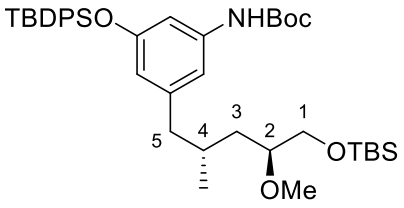
169

The alcohol **168** (5.35 g, 9.27 mmol, 1.0 eq.) was dissolved under argon atmosphere in CH_2Cl_2 (380 mL), cooled down to 0 °C and treated with 2,6-lutidine (1.07 mL, 9.27 mmol, 1.0 eq.) and then TBSOTf (2.13 mL, 9.27 mmol, 1.0 eq.). After 20 min the reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 15 : 1 to 4 : 1) and the protected alcohol **169** (4.65 g, 6.86 mmol, 74%) was obtained as a colorless foam.

The analytical data are in accordance with the reported in the literature.[136]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.72 – 7.70 (m, 4H, SiPh), 7.41 – 7.35 (m, 6H, SiPh), 6.78 (s, 1H, Ph), 6.66 (m, 1H, Ph), 6.27 (bs, 1H, NH), 6.15 (m, 1H, Ph), 3.69 – 3.64 (m, 1H, 2-H), 3.52 (dd, *J* = 9.9, 3.4 Hz, 1H, 1-Ha), 3.31 (dd, *J* = 9.9, 7.6 Hz, 1H, 1-Hb), 2.40 (dd, *J* = 13.3, 6.0 Hz, 1H, 5-Ha), 2.24 (s, 1H, 2-OH), 2.13 (dd, *J* = 13.3, 8.4 Hz, 1H, 5-Hb), 1.85 – 1.72 (m, 1H, 4-H), 1.48 (s, 9H,*t*-Bu), 1.37 – 1.30 (m, 1H, 3-Ha), 1.08 (s, 9H, Si*t*-Bu), 0.97 – 0.93 (m, 1H, 3-Hb), 0.90 (s, 9H, Si*t*-Bu), 0.67 (d, *J* = 6.6 Hz, 3H, 4-Me), 0.07 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 155.9 (s, C-Ar), 152.6 (s, NCOO), 142.9 (s, C-Ar), 139.0 (s, C-Ar), 135.6 (d, C-Ar), 133.1 (s, C-Ar), 129.9 (d, C-Ar), 127.9 (d, C-Ar), 115.6 (d, C-Ar), 112.3 (d, C-Ar), 107.6 (d, C-Ar), 80.5 (s, *t*-Bu), 69.7 (d, C-2), 68.0 (t, C-1), 44.3 (t, C-5), 39.7 (t, C-3), 31.0 (d, C-4), 28.5 (q, *t*-Bu), 26.7 (q, Si*t*-Bu), 26.0 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu), 18.7 (q, 4-Me), 18.4 (s, Si*t*-Bu), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{39}H_{59}NO_5NaSi_2]^+$: 700.3830, found: 700.3827; **$[\alpha]^{20}_D$** = −2.5° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −4.7°, *c* = 1.8, CH_2Cl_2).[136]

(2*S*,4*R*)-5-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)-phenyl]-4-methyl-1-(*tert*-butyldemethylsiloxy)-2-methoxypentane (170)



$C_{40}H_{61}NO_5Si_2$
Mol. Wt.: 692.10 g/mol

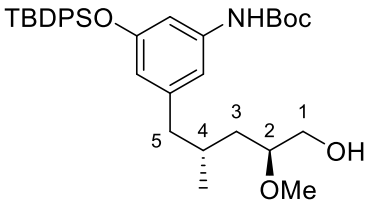
170

The alcohol **169** (4.0 g, 5.90 mmol, 1.0 eq.) was dissolved under argon atmosphere in CH_2Cl_2 (120 mL) and 1,8-bis(dimethylamino)-naphthalene (4.42 g, 20.7 mmol, 3.5 eq.), and Me_3OBF_4 (2.18 g, 14.8 mmol, 2.5 eq.) were added. After 1 h the reaction was terminated by addition of water. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 15 : 1 to 4 : 1) to give the methylated alcohol **170** (3.47 g, 5.0 mmol, 85%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[136]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.72 – 7.69 (m, 4H, SiPh), 7.43 – 7.33 (m, 6H, SiPh), 6.78 (s, 1H, Ph), 6.74 (m, 1H, Ph), 6.38 (bs, 1H, NH), 6.18 (m, 1H, Ph), 3.62 (dd, *J* = 10.5, 5.8 Hz, 1H, 1-Ha), 3.50 (dd, *J* = 10.5, 4.7 Hz, 1H, 1-Hb), 3.39 (s, 3H, OMe), 3.25 – 3.19 (m, 1H, 2-H), 2.44 (dd, *J* = 13.2, 5.4 Hz, 1H, 5-Ha), 2.09 (dd, *J* = 13.1, 9.1 Hz, 1H, 5-Hb), 1.74 – 1.66 (m, 1H, 4-H), 1.48 (s, 9H, *t*-Bu), 1.42 – 1.35 (m, 1H, 3-Ha), 1.08 (s, 9H, Si*t*-Bu), 0.98 – 0.93 (m, 1H, 3-Hb), 0.93 (s, 9H, Si*t*-Bu), 0.66 (d, *J* = 6.5 Hz, 3H, 4-Me), 0.05 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 155.9 (s, C-Ar), 152.6 (s, NCOO), 143.0 (s, C-Ar), 139.0 (s, C-Ar), 135.6 (d, C-Ar), 133.1 (s, C-Ar), 129.9 (d, C-Ar), 127.8 (d, C-Ar), 115.5 (d, C-Ar), 112.3 (d, C-Ar), 107.6 (d, C-Ar), 80.3 (s, *t*-Bu), 80.0 (d, C-2), 66.0 (t, C-1), 58.1 (q, OMe), 44.3 (t, C-5), 39.1 (t, C-3), 31.2 (d, C-4), 28.4 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 26.0 (q, Si*t*-Bu), 19.5 (s, Si*t*-Bu), 19.0 (q, 4-Me), 18.4 (s, Si*t*-Bu), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{39}H_{59}NO_5NaSi_2]^+$: 586.2965, found: 586.2969; $[\alpha]^{20}_D$ = −7.5° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −7.4°, *c* = 1.0, $CHCl_3$).[136]

(2*S*,4*R*)-5-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)-phenyl]-4-methyl-2-methoxypentane (171)



$C_{34}H_{47}NO_5Si$
Mol. Wt.: 577.84 g/mol

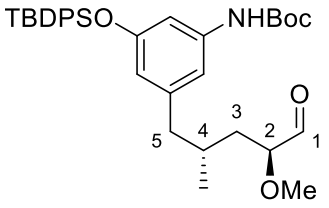
171

The alcohol **170** (3.40 g, 4.91 mmol, 1.0 eq.) was dissolved under argon atmosphere in CH_2Cl_2 (35 mL) and treated with MeCN (35 mL) and $LiBF_4$ (1.0 M solution in acetonitrile) (14.7 mL, 14.7 mmol, 3.0 eq.). After 48 h the reaction was terminated by addition of water. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 10 : 1 to 2 : 1) followed by preparative HPLC (C18 column, H_2O : MeOH = 80 : 20 (5 min), gradient H_2O : MeOH = 80 : 20 to 0 : 100 (85 min, 15 mL/min, t_R = 85.0 min) to give the methylated alcohol **171** (2.13 g, 3.68 mmol, 75%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[136]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.73 – 7.68 (m, 4H, SiPh), 7.44 – 7.33 (m, 6H, SiPh), 6.78 (s, 1H, Ph), 6.67 (m, 1H, Ph), 6.30 (bs, 1H, NH), 6.12 (m, 1H, Ph), 3.64 – 3.56 (m, 1H, 1-Ha), 3.41 – 3.35 (m, 1H, 1-Hb), 3.31 (s, 3H, OMe), 3.28 – 3.21 (m, 1H, 2-H), 2.39 (dd, *J* = 13.3, 5.7 Hz, 1H, 5-Ha), 2.08 (dd, *J* = 13.3, 8.6 Hz, 1H, 5-Hb), 1.91 (bs, 1H, 1-OH), 1.64 – 1.58 (m, 1H, 4-H), 1.48 (s, 9H, *t*-Bu), 1.53 – 1.46 (m, 1H, 3-Ha), 1.08 (s, 9H, Si*t*-Bu), 1.05 – 1.00 (m, 1H, 3-Hb), 0.65 (d, *J* = 6.6 Hz, 3H, 4-Me; **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 156.0 (s, C-Ar), 152.7 (s, NCOO), 142.8 (s, C-Ar), 139.1 (s, C-Ar), 135.6 (d, C-Ar), 133.1 (s, C-Ar), 129.9 (d, C-Ar), 127.9 (d, C-Ar), 115.5 (d, C-Ar), 112.3 (d, C-Ar), 107.7 (d, C-Ar), 80.5 (s, *t*-Bu), 79.6 (d, C-2), 64.3 (t, C-1), 57.1 (q, OMe), 44.0 (t, C-5), 38.0 (t, C-3), 31.4 (d, C-4), 28.5 (q, *t*-Bu), 26.6 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu), 19.5 (q, 4-Me); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{34}H_{47}NO_5NaSi]^+$: 600.3121, found: 600.3120; **$[\alpha]^{20}_D$** = +6.0° (c = 1.0, CH_2Cl_2; Lit. $[\alpha]^{20}_D$ = +5.0°, *c* = 1.1, CH_2Cl_2).[136]

(2*S*,4*R*)-5-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)-phenyl]-4-methyl-1-(*tert*-butyldemethylsiloxy)-2-methoxy-1-pentanal (153)



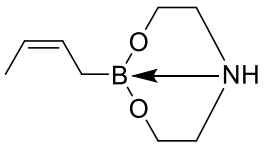
$C_{34}H_{45}NO_5Si$
Mol. Wt.: 575.82 g/mol

153

The alcohol **171** (0.087 g, 0.15 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (12 mL), cooled down to 0 °C and treated with $NaHCO_3$ (0.015 g, 0.18 mmol, 1.2 eq.) and DMP reagent (0.077 g, 0.18 mmol, 1.2 eq.). After 1 h at RT the reaction was terminated by addition of an aqueous Na_2SO_3 solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure providing the aldehyde **153** (0.085 g, 0.15 mmol, 99%) as a colorless foam. The product was used directly in the next step without further characterization.

(*Z*)-Crotylboronate (172)

(*Z*)-Crotylboronate **172** was prepared according to the procedure of White et al.[172]

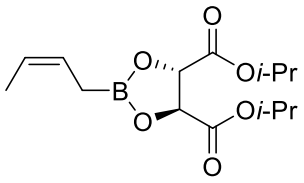


$C_8H_{16}BNO_2$
Mol. Wt.: 169.03 g/mol

172a

Potassium *tert*-butoxide (2.0 g, 18.0 mmol, 1.1 eq.) was suspended under argon atmosphere in THF (10 mL) and cooled down to −78 °C. *cis*-Butene (1.45 mL, 16.4 mmol, 1.0 eq.) was added dropwise via cannula to the reaction mixture, followed by *n*-BuLi (2.5 M in hexanes) (5.6 mL, 14.0 mmol, 0.8 eq.). The mixture was stirred at −78 °C for 1 h, then warmed up to −25 °C and stirred at this temperature for 45 min. The reaction mixture was again cooled down to −78 °C and triisopropyl borate (3.14 mL, 14.0 mmol, 0.8 eq.) was added dropwise. The mixture was stirred at −78 °C for 30 min and the reaction was terminated with 1 M hydrochloric acid (5.0 mL). While vigorous stirring additional 1 M hydrochloric acid (20 mL) was added and the solution was extracted three times with EtOAc. The combined organic

phases were added under argon atmosphere to powdered 4Å molecular sieves and diethanolamine (1.1 mL, 12.0 mmol, 0.7 eq.). The mixture was stirred for 3 h, filtered and washed with EtOAc. Removal of the solvent under reduced pressure provided (*Z*)-crotylaminoboronate complex **172a** as a white solid which was recrystallized from a dichloromethane-diethyl ether mixture to give colorless crystals. The obtained material was stored under argon atmosphere.

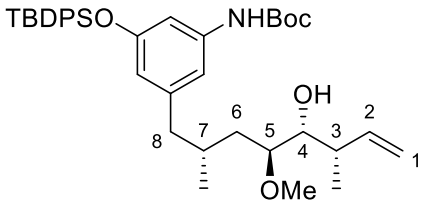


$C_{14}H_{23}BO_6$
Mol. Wt.: 298.14 g/mol

172

(*S*,*S*)-Diisopropyl tartrate (0.1 mL, 0.47 mmol, 1.0 eq.) was added to a rapidly stirred suspension of the (*Z*)-crotylaminoboronate complex **172a** (0.08 g, 0.47 mmol, 1.0 eq.) in Et_2O (1.5 mL) under argon atmosphere at RT. The mixture was stirred for 5 min, brine (1.5 mL) was added and the rapid stirring was continued for 5 min. The mixture was extracted three times with Et_2O and the combined organic extracts were dried over $MgSO_4$. Removal of the solvent under reduced pressure afforded the product **172** as a colorless oil (0.14 g, 0.47 mmol) which was used immediately for the preparation of the western fragment **148**.

(3*S*,4*R*,5*S*,7*R*)-8-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)-phenyl]-4-hydroxy-5-methoxy-3,7-dimethyl-oct-2-en (148)



$C_{38}H_{53}NO_5Si$
Mol. Wt.: 631.93 g/mol

148

(*Z*)-Crotylboronate **172** (0.112 g, 0.38 mmol, 2.5 eq.) was dissolved under argon atmosphere in toluene (1.5 mL). Then, 4Å molecular sieves (0.063 g) were added, the reaction mixture was stirred at RT for 20 min and then cooled down to −78 °C. The aldehyde **153** (0.085 g, 0.15 mmol, 1.0 eq.) was dissolved in toluene (0.7 mL) and slowly added to the reaction mixture. The reaction was terminated after 20 h by addition of an aqueous NaCl solution (1.0

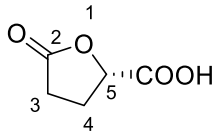
M). The reaction mixture was warmed up to RT and stirred for 1 h. The molecular sieves were removed by filtration through Celite™ and the aqueous phase was extracted three times with Et_2O. The combined organic phases were washed with aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 10 : 1 to 4 : 1) providing the alkene **148** (0.076 g, 0.12 mmol, 80%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.73 – 7.68 (m, 4H, SiPh), 7.42 – 7.34 (m, 6H, SiPh), 6.77 (s, 1H, Ph), 6.65 (m, 1H, Ph), 6.24 (bs, 1H, NH), 6.16 (m, 1H, Ph), 5.60 (ddd, *J* = 17.2, 10.3, 8.6 Hz 1H, 2-H), 5.03 (d, *J* = 10.1 Hz, 1H, 1-Ha), 4.99 (d, *J* = 3.0 Hz 1H, 1-Hb), 3.58 (d, *J* = 8.9 Hz 1H, 4-H), 3.29 (s, 3H, OMe), 3.23 – 3.15 (m, 1H, 5-H), 2.42 (dd, *J* = 13.3, 5.5 Hz, 1H, 8-Ha), 2.27 – 2.15 (m, 1H, 3-H), 2.09 (bs, 1H, 4-OH), 2.09 – 2.06 (m, 1H, 8-Hb), 1.71 – 1.63 (m, 1H, 7-H), 1.56 – 1.52 (m, 1H, 6-Ha), 1.48 (s, 9H, *t*-Bu), 1.12 (d, *J* = 6.6 Hz, 3H, 3-Me), 1.08 (s, 9H, Si*t*-Bu), 1.07 – 1.02 (m, 1H, 6-Hb), 0.58 (d, *J* = 6.5 Hz, 3H, 7-Me); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 155.9 (s, C-Ar), 152.6 (s, NCOO), 143.1 (s, C-Ar), 140.1 (d, C-2), 139.0 (s, C-Ar), 135.7 (d, C-Ar), 133.1 (s, C-Ar), 129.9 (d, C-Ar), 127.8 (d, C-Ar), 115.6 (t, C-1),), 115.3 (d, C-Ar), 112.3 (d, C-Ar), 107.7 (d, C-Ar), 80.4 (s, *t*-Bu), 80.2 (d, C-5), 73.5 (d, C-4), 57.1 (q, OMe), 44.7 (t, C-8), 40.5 (d, C-3), 34.8 (t, C-6), 30.9 (d, C-7), 28.5 (q, *t*-Bu), 26.7 (q, Si*t*-Bu), 19.6 (s, Si*t*-Bu), 18.6 (q, 7-Me), 17.5 (q, 3-Me); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{38}H_{53}NO_5NaSi]^+$: 654.3591, found: 654.3590; **$[\alpha]^{20}_D$** = −17.6° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −17.8°, *c* = 1.0, $CHCl_3$).[135]

3.6 Synthesis of the Eastern Fragments

(*S*)-5-Oxotetrahydrofuran-2-carboxylic acid (173a)



$C_5H_6O_4$
Mol. Wt.: 130.10 g/mol

173a

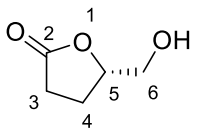
L-Glutamic acid (**158**-L) (19.0 g, 129 mmol, 1.0 eq.) was dissolved in water (140 mL), treated with HCl (2 M, 85 mL) and cooled down to −10 °C. $NaNO_2$ (10.7 g, 155 mmol, 1.2 eq.) was dissolved in water (85 mL) and slowly added to the reaction mixture over a period of 3 h. The

reaction mixture was warmed up to RT and stirring was continued for 12 h. The aqueous phase was extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The acid **173a** (8.95 g, 68.8 mmol, 53%) was obtained as a colorless solid.

The analytical data are in accordance with the reported in the literature.[173]

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 5.00 (dd, J = 7.8, 5.0 Hz, 1H, H-5), 2.71 – 2.54 (m, 2H, H-3), 2.54 – 2.41 (m, 1H, H-4a), 2.38 – 2.25 (m, 1H, H-4b); **HRMS-ESI** (m/z): $[M+Na]^+$ calcd for $[C_5H_6O_4Na]^+$: 153.0164, found: 153.0161; **M.p.**: 70 °C; **$[\alpha]^{20}_D$** = +14.0° (c = 1.0, MeOH; Lit. $[\alpha]^{20}_D$ = +15.6°, c = 2.0, EtOH).[173]

(*S*)-5-(Hydroxymethyl)-2-oxotetrahydrofuran (red-173a)



$C_5H_8O_3$
Mol. Wt.: 116.12 g/mol

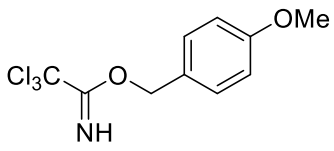
red-173a

The acid **173a** (8.95 g, 68.8 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in THF (240 mL), cooled down to 0 °C and treated slowly with $BH_3 \cdot SMe_2$ (9.8 mL, 103.2 mmol, 1.5 eq.). The reaction mixture was warmed to RT and stirred at RT for additional 3 h. The reaction was terminated by addition of MeOH. The solvent was removed under reduced pressure to give the lactone **red-173a** (7.75 g, 66.7 mmol, 97%) as a colorless oil.

The analytical data are in accordance with the reported in the literature.[173]

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 4.67 – 4.56 (m, 1H, H-5), 3.78 (dd, J = 11.9, 3.1 Hz, 1H, H-6a), 3.59 (dd, J = 11.9, 4.5 Hz, 1H, H-6b), 2.61 – 2.52 (m, 2H, H-3), 2.37 – 2.03 (m, 2H, H-4); **$[\alpha]^{20}_D$** = +44.5° (c = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = +31.3°, c = 2.92, EtOH).[173]

4-Methoxybenzyl-2,2,2-trichloroacetimidate (179)



$C_{10}H_{10}Cl_3NO_2$
Mol. Wt.: 282.54 g/mol

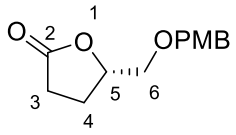
179

A dried 200 mL round bottom flask was charged with sodium hydride (60% dispersion in mineral oil, 0.64 g, 16.0 mmol). Et_2O (50 mL) was added followed by 4-methoxy-benzyl alcohol (20.0 mL, 160 mmol) which was added slowly over a period of 15 min. The mixture was stirred at RT for 30 min and then cooled down to 0 °C. Trichloroacetonitrile (17.7 mL, 176 mmol) was added dropwise over a period of 20 min. After 1 h the reaction was warmed up to RT and stirred for 45 min. The reaction was then concentrated *in vacuo* to give a dark orange oil. This crude residue was treated with a mixture of pentane and MeOH (170 mL, pentane : MeOH = 275 : 1) and stirring was continued for 30 min. The heterogeneous mixture was filtered through a plug of Celite^{TM} and the filtrate was concentrated *in vacuo* to afford the trichloroacetimidate **179** (43.0 g, 152 mmol, 95%) as an orange oil.

The analytical data are in accordance with the reported in the literature.[144]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 8.36 (s, 1H, NH), 7.37 (d, J = 8.8 Hz, 2H, , Ar), 6.91 (d, J = 8.8 Hz, 2H, Ar), 5.27 (s, 2H, CH_2), 3.82 (s, 3H, OMe).

(*S*)-5-(4-Methoxybenzyloxymethyl)-2-oxotetrahydrofuran (174a)



$C_{13}H_{16}O_4$
Mol. Wt.: 236.27 g/mol

174a

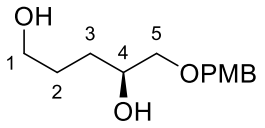
The alcohol **red-173a** (1.0 g, 8.61 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (50 mL) under nitrogen atmosphere and treated with PMB-trichloroacetimidate (**179**) (9.73 g, 34.5 mmol, 4.0 eq.) and CSA (0.80 g, 3.45 mmol, 0.4 eq.). After 24 h the reaction was terminated by addition of an aqueous $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 10 : 1 to 2 : 1) and the PMB-protected alcohol **174a** (1.2 g, 5.1 mmol, 60%) was obtained as a colorless oil.

The analytical data are in accordance with the reported in the literature.[174]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.26 (d, J = 8.7 Hz, 2H, PMB), 6.90 (d, J = 8.7 Hz, 2H, PMB), 4.69 – 4.64 (m, 1H, H-5), 4.55 – 4.44 (m, 2H, PMB), 3.82 (s, 3H, PMB), 3.66 (dd, J = 10.8, 3.4 Hz, 1H, H-6a), 3.57 (dd, J = 10.7, 4.2 Hz, 1H, H-6b), 2.67

– 2.58 (m, 1H, H-3a), 2.53 – 2.44 (m, 1H, H-3b), 2.34 – 2.24 (m, 1H, H-4a), 2.17 – 2.06 (m, 1H, H-4b); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 177.5 (s, C-2), 159.5 (s, PMB), 129.4 (d, PMB), 114.0 (d, PMB), 79.2 (d, C-5), 73.4 (t, PMB), 71.4 (t, C-6), 55.4 (q, PMB), 28.5 (t, C-3), 24.3 (t, C-4); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{13}H_{16}O_4Na]^+$: 259.0946, found: 259.0947; **$[\alpha]^{20}_D$** = +9.0° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = +10.6°, *c* = 1.0, $CHCl_3$).[174]

(*S*)-5-(4-Methoxybenzyloxy)pentan-1,4-diol (175a)



$C_{13}H_{20}O_4$
Mol. Wt.: 240.30 g/mol

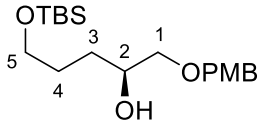
175a

The lactone **174a** (1.2 g, 5.08 mmol, 1.0 eq.) was dissolved in THF (140 mL) under nitrogen atmosphere, cooled down to 0 °C and treated with $LiAlH_4$ portionwise (0.21 g, 5.59 mmol, 1.1 eq.). After 30 min the reaction was terminated by addition of water. After another 10 min the precipitate was filtered through Celite^{TM}, the filtrate was diluted with water and extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 1 : 1 to 1 : 5) to give the diol **175a** (0.95 g, 3.95 mmol, 78%) as a colorless oil.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.29 (d, *J* = 8.5 Hz, 2H, PMB), 6.91 (d, *J* = 8.7 Hz, 2H, PMB), 4.51 (s, 2H, PMB), 3.91 – 3.85 (m, 1H, H-4), 3.84 (s, 3H, PMB), 3.74 – 3.63 (m, 2H, H-1), 3.50 (dd, *J* = 9.4, 3.3 Hz, 1H, H-5a), 3.35 (dd, *J* = 9.4, 8.0 Hz, 1H, H-5b), 2.84 (bs, 1H, 4-OH), 2.42 (bs, 1H, 1-OH), 1.76 – 1.67 (m, 2H, H-2), 1.66– 1.58 (m, 1H, H-3a), 1.56– 1.47 (m, 1H, H-3b); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 159.5 (s, PMB), 129.6 (d, PMB), 114.0 (d, PMB), 74.3 (d, C-4), 73.2 (t, PMB), 70.5 (t, C-5), 63.0 (t, C-1), 55.4 (q, PMB), 30.3 (t, C-2), 29.3 (t, C-3); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{13}H_{20}O_4Na]^+$: 263.1259, found: 263.1257; **$[\alpha]^{20}_D$** = +1.8° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = +1.9°, *c* = 1.0, $CHCl_3$).[135]

(*S*)-5-(*tert*-Butyldimethylsilyloxy)-1-(4-methoxybenzyloxy)pentan-2-ol (176a)



$C_{19}H_{34}O_4Si$
Mol. Wt.: 354.56 g/mol

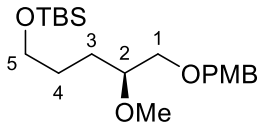
176a

The diol **175a** (1.0 g, 4.16 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (30 mL) under nitrogen atmosphere, cooled down to 0 °C and treated with imidazole (0.57 g, 8.32 mmol, 2.0 eq.) followed by TBSCl (0.69 g, 4.6 mmol, 1.1 eq.). The reaction mixture was warmed up to RT and stirred for 1 h. The reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 5 : 1 to 2 : 1) to give the protected alcohol **176a** (1.33 g, 3.7 mmol, 90%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.30 (d, J = 8.7 Hz, 2H, PMB), 6.92 (d, J = 8.7 Hz, 2H, PMB), 4.52 (s, 2H, PMB), 3.94 – 3.84 (m, 1H, H-2), 3.84 (s, 3H, PMB), 3.73 – 3.63 (m, 2H, H-5), 3.51 (dd, J = 9.4, 3.7 Hz, 1H, H-1a), 3.37 (dd, J = 9.4, 7.4 Hz, 1H, H-1b), 2.92 (bs, 1H, 2-OH), 1.77 – 1.48 (m, 2H, H-4), 1.77 – 1.48 (m, 2H, H-4), 0.93 (s, 9H, Si*t*-Bu), 0.09 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 159.4 (s, PMB), 129.5 (s, PMB), 114.0 (d, PMB), 74.4 (d, C-2), 73.1 (t, PMB), 70.4 (t, C-1), 63.4 (t, C-5), 55.4 (q, PMB), 30.4 (t, C-4), 29.1 (t, C-3), 26.1 (q, Si*t*-Bu), 18.5 (s, Si*t*-Bu), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{19}H_{34}O_4NaSi]^+$: 377.2124, found: 377.2126; $[\alpha]^{20}_D$ = −2.0° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −2.3°, *c* = 1.0, $CHCl_3$).[135]

(*S*)-5-(*tert*-Butyldimethylsilyloxy)-1-(4-methoxybenzyloxy)-2-methoxypentan (177a)



$C_{20}H_{36}O_4Si$
Mol. Wt.: 368.59 g/mol

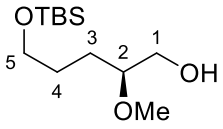
177a

The alcohol **176a** (1.2 g, 3.39 mmol, 1.0 eq.) was dissolved in THF (40 mL) under nitrogen atmosphere, cooled down to 0 °C and treated with NaH (0.12 g, 5.08 mmol, 1.5 eq.) followed by MeI (0.23 mL, 3.72 mmol, 1.1 eq.). The reaction mixture was warmed up to RT and stirred for 24 h. The reaction was terminated by addition of water. The phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 5 : 1 to 2 : 1) to give the protected alcohol **177a** (1.18 g, 3.19 mmol, 94%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.26 (d, J = 8.6 Hz, 2H, PMB), 6.87 (d, J = 8.7 Hz, 2H, PMB), 4.48 (s, 2H, PMB), 3.80 (s, 3H, PMB), 3.63 – 3.57 (m, 2H, H-5), 3.45 (d, J = 4.9 Hz, 2H, H-1), 3.40 (s, 3H, Me), 3.39 – 3.34 (m, 1H, H-2), 1.60 – 1.49 (m, 2H, H-4), 0.89 (s, 9H, Si*t*-Bu), 0.04 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 159.3 (s, PMB), 130.6 (s, PMB), 129.4 (d, PMB), 113.9 (d, PMB), 80.1 (d, C-2), 73.1 (t, PMB), 71.9 (t, C-1), 63.4 (t, C-5), 57.6 (q, OMe), 55.4 (q, PMB), 28.8 (t, C-4), 27.9 (t, C-3), 26.1 (q, Si*t*-Bu), 18.5 (s, Si*t*-Bu), -5.1 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{20}H_{36}O_4NaSi]^+$: 391.2281, found: 391.2281; $\mathbf{[\alpha]^{20}_D}$ = −6.0° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −6.0°, *c* = 1.0, $CHCl_3$).[135]

(*S*)-5-(*tert*-Butyldimethylsilyloxy)-2-methoxypentan-1-ol (178a)



$C_{12}H_{28}O_3Si$
Mol. Wt.: 248.44 g/mol

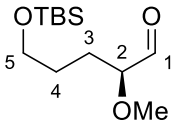
178a

The alcohol **177a** (1.18 g, 3.19 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (90 mL), cooled down to 0 °C and treated with water (9 mL) and DDQ (0.87 g, 3.84 mmol, 1.2 eq.). The reaction mixture was warmed up to RT and stirred for 3 h. The reaction was terminated by addition of an aqueous $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with aqueous NaCl solution, dried over $MgSO_4$ and removed under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 15 : 1 to 5 : 1) and the deprotected alcohol **178a** (0.7 g, 2.82 mmol, 89%) was obtained as a colorless oil.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 3.71 – 3.65 (m, 1H, H-1a), 3.63 – 3.60 (m, 2H, H-5), 3.52 – 3.45 (m, 1H, H-1b), 3.40 (s, 3H, OMe), 3.32 – 3.26 (m, 1H, H-2), 2.08 – 2.05 (m, 1H, OH), 1.66 – 1.47 (m, 2H, H-4), 0.88 (s, 9H, Si*t*-Bu), 0.04 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 81.5 (d, C-2), 64.1 (t, C-1), 63.2 (t, C-5), 57.2 (q, OMe), 28.6 (t, C-4), 26.7 (t, C-3), 26.1 (q, Si*t*-Bu), 18.5 (s, Si*t*-Bu), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{12}H_{28}O_3NaSi]^+$: 271.1705, found: 271.1707; **$[\alpha]^{20}_D$** = +20.0° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = +20.0°, *c* = 1.0, $CHCl_3$).[135]

(*S*)-5-(*tert*-Butyldimethylsilyloxy)-2-methoxypentan-1-al (157a)

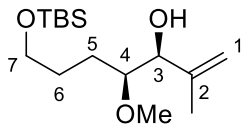


$C_{12}H_{26}O_3Si$
Mol. Wt.: 246.42 g/mol

157a

The alcohol **178a** (15.0 mg, 60 µmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (5 mL), cooled down to 0 °C and treated with $NaHCO_3$ (6.0 mg, 72 µmol, 1.2 eq.) and DMP reagent (31.0 mg, 72 µmol, 1.2 eq.). After stirring at RT for 1 h the reaction was terminated by addition of an aqueous Na_2SO_3 solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure providing the aldehyde **157a** (14.7 mg, 59 µmol, 99%) as a colorless solid. The product was used for the next step without further characterization.

(3*S*,4*S*)-7-(*tert*-Butyldimethylsilyloxy)-4-methoxy-2-methylhept-1-en-3-ol (149)



$C_{15}H_{32}O_3Si$
Mol. Wt.: 288.50 g/mol

149

1st Method:

The aldehyde **157a** (14.7 mg, 59 µmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (1 mL), cooled down to 0 °C and treated with $MgBr_2 \cdot Et_2O$ (37 mg, 145 µmol, 2.4

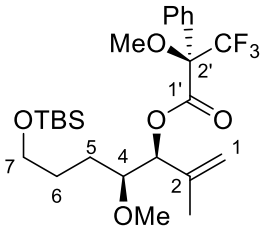
eq.). The reaction mixture was stirred at 0 °C for 30 min and then cooled down to −78 °C followed by a slow addition of isopropenylmagnesium bromide (0.5 M in THF) (0.3 mL, 150 µmol, 2.5 eq.). After 3 h the reaction mixture was warmed up to RT, stirred for additional 30 min and terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 5 : 1 to 2 : 1) providing the product **149** (9.0 mg, 31 µmol, 53%, *d.r. syn* : *anti* > 95:5) as a colorless oil.

2nd Method:

Isopropenylmagnesium bromide (0.5 M in THF) (3.2 mL, 1.6 mmol, 2.0 eq.) was diluted in THF (8 mL), cooled down to −78 °C and transferred via cannula to a cooled solution of aldehyde **157a** (196 mg, 0.8 mmol, 1.0 eq.) in THF (10 mL). After 30 min of stirring at −78 °C the reaction was terminated by addition of $NaHCO_3$ and the mixture was diluted with EtOAc. The phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 5 : 1 to 2 : 1) providing the product **149** (130 mg, 0.45 mmol, 56%, d.r. *syn* : *anti* > 95:5) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 5.03 – 5.00 (m, 1H, H-1a), 4.95 – 4.91 (m, 1H, H-1b), 3.94 (d, *J* = 6.8 Hz, 1H, H-3), 3.62 – 3.58 (m, 2H, H-7), 3.42 (s, 3H, OMe), 3.27 – 3.23 (m, 1H, H-4), 2.63 (d, 1H, 3-OH), 1.75 (s, 3H, 2-Me), 1.63 – 1.55 (m, 2H, H-5), 1.63 – 1.55 (m, 2H, H-6), 0.89 (s, 9H, Si*t*-Bu), 0.04 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 144.4 (s, C-2), 114.1 (t, C-1), 82.1 (d, C-4), 77.5 (d, C-3), 63.2 (t, C-7), 58.0 (q, OMe), 28.2 (t, C-6), 26.4 (t, C-5), 26.1 (q, Si*t*-Bu), 18.5 (s, Si*t*-Bu), 18.0 (q, 2-Me), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{15}H_{32}O_3NaSi]^+$: 311.2018, found: 311.2011; $\mathbf{[\alpha]^{20}_D}$ = +22.5° (*c* = 2.0, CH_2Cl_2).

(*R*)-Mosher ester (180a)



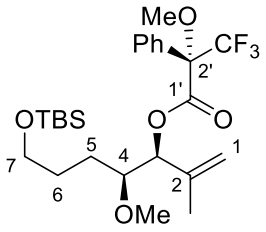
$C_{25}H_{39}F_3O_5Si$
Mol. Wt.: 504.66 g/mol

(*R*)-**180a**

The alcohol **149** (7.3 mg, 25.3 µmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (1.0 mL), cooled down to 0 °C and treated with Et_3N (14 µl, 101 µmol, 4.0 eq.) and DMAP (0.3 mg, 2.5 µmol, 0.1 eq.) followed by (*S*)-Mosher acid chloride (7.7 mg, 30.4 µmol, 1.2 eq.). The reaction mixture was warmed up to RT and stirred for 4 h. The reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 20 : 1) to give the ester (*R*)-**180a** (10.7 mg, 21.2 µmol, 84%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.55 – 7.53 (m, 2H, Ph), 7.41 – 7.37 (m, 3H, H-Ph), 5.41 (d, *J* = 8.0 Hz, 1H, H-3), 5.13 (s, 1H, H-1a), 5.06 – 5.02 (m, 1H, H-1b), 3.60 – 3.54 (m, 2H, H-7), 3.56 (s, 3H, 2'-OMe), 3.36 – 3.30 (m, 1H, H-4), 3.03 (s, 3H, 4-OMe), 1.76 (s, 3H, 2-Me), 1.63 – 1.50 (m, 2H, H-5), 1.63 – 1.50 (m, 2H, H-6), 0.87 (s, 9H, Si*t*-Bu), 0.02 (s, 6H, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{25}H_{39}O_5F_3NaSi]^+$: 527.2417, found: 527.2420.

(*S*)-Mosher ester (180b)



$C_{25}H_{39}F_3O_5Si$
Mol. Wt.: 504.66 g/mol

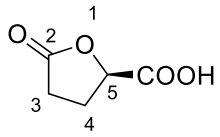
(*S*)-**180b**

The alcohol **149** (8.8 mg, 30.5 µmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (1.5 mL), cooled down to 0 °C and treated with Et_3N (17 µl, 122 µmol, 4.0 eq.) and

DMAP (0.4 mg, 3.7 µmol, 0.1 eq.) followed by (*R*)-Mosher acid chloride (9.3 mg, 36.7 µmol, 1.2 eq.). The reaction mixture was warmed up to RT and stirred for 4 h. The reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 20 : 1) to give the ester (*S*)-**180b** (12.7 mg, 25.2 µmol, 82%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.57 – 7.55 (m, 2H, Ph), 7.41 – 7.37 (m, 3H, H-Ph), 5.35 (d, *J* = 8.4 Hz, 1H, H-3), 5.08 (s, 1H, H-1a), 5.03 – 4.98 (m, 1H, H-1b), 3.63 – 3.53 (m, 2H, H-7), 3.57 (s, 3H, 2'-OMe), 3.45 – 3.39 (m, 1H, H-4), 3.36 (s, 3H, 4-OMe), 1.80 – 1.40 (m, 2H, H-5), 1.80 – 1.40 (m, 2H, H-6), 1.63 (s, 3H, 2-Me), 0.88 (s, 9H, Si*t*-Bu), 0.03 (s, 6H, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{25}H_{39}O_5F_3NaSi]^+$: 527.2417, found: 527.2422.

(*R*)-5-Oxotetrahydrofuran-2-carboxylic acid (173b)



173b

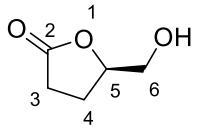
$C_5H_6O_4$
Mol. Wt.: 130.0990 g/mol

D-Glutamic acid (**158**-D) (21.0 g, 143 mmol, 1.0 eq.) was dissolved in water (143 mL), treated with HCl (2 M, 85 mL) and cooled down to −10 °C. $NaNO_2$ (11.8 g, 171 mmol, 1.2 eq.) was dissolved in water (85 mL) and slowly added to the reaction mixture over a period of 3 h. The reaction mixture was warmed to RT and stirred for 12 h. The aqueous phase was extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The acid **173b** (10.8 g, 83.0 mmol, 58%) was obtained as a colorless solid.

The analytical data are in accordance with the reported in the literature.[143]

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 5.00 (dd, *J* = 7.8, 5.0 Hz, 1H, H-5), 2.71 – 2.54 (m, 2H, H-3), 2.54 – 2.41 (m, 1H, H-4a), 2.38 – 2.25 (m, 1H, H-4b); **HRMS-ESI** (*m*/*z*): $[M-H]^-$ calcd for $[C_5H_5O_4]^-$: 129.0188, found: 129.0188; **M.p.** = 71 °C (Lit. 71 °C) [143]; **$[\alpha]^{20}_D$** = −13.0° (c = 1.0, MeOH; Lit. $[\alpha]^{20}_D$ = −15.5°, *c* = 2.0, EtOH).[143]

(*R*)-5-(Hydroxymethyl)-2-oxotetrahydrofuran (red-173b)



$C_5H_8O_3$
Mol. Wt.: 116.12 g/mol

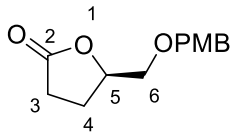
red-173b

The acid **173b** (9.7 g, 74.6 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in THF (160 mL), cooled down to 0 °C and treated slowly with $BH_3 \cdot SMe_2$ (10.6 mL, 111.5 mmol, 1.5 eq.). The reaction mixture was warmed up to RT and stirred at RT for additional 3 h. The reaction was terminated by addition of MeOH. The solvent was removed under reduced pressure to give the lactone **red-173b** (8.45 g, 72.8 mmol, 98%) as a colorless oil.

The analytical data are in accordance with the reported in the literature.[135,143]

^{1}H NMR (400 MHz, CD_3OD, CD_2HOD = 3.31 ppm) δ (ppm): 4.67 – 4.56 (m, 1H, H-5), 3.78 (dd, J = 11.9, 3.1 Hz, 1H, H-6a), 3.59 (dd, J = 11.9, 4.5 Hz, 1H, H-6b), 2.61 – 2.52 (m, 2H, H-3), 2.37 – 2.03 (m, 2H, H-4); **$[\alpha]^{20}_D$** = −44.0° (c = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −33.0°, c = 3.0, EtOH).[143]

(*R*)-5-(4-Methoxybenzyloxymethyl)-2-oxotetrahydrofuran (174b)



$C_{13}H_{16}O_4$
Mol. Wt.: 236.23 g/mol

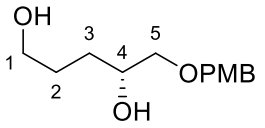
174b

The alcohol **red-173b** (1.0 g, 8.61 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (50 mL) under nitrogen atmosphere and treated with PMB-trichloroacetimidate (**179**) (9.73 g, 34.5 mmol, 4.0 eq.) and CSA (0.80 g, 3.45 mmol, 0.4 eq.). After 24 h the reaction was terminated by addition of an aqueous $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 10 : 1 to 2 : 1) and the PMB-protected alcohol **174b** (1.52 g, 6.5 mmol, 75%) was obtained as a colorless oil.

The analytical data are in accordance with the reported in the literature.[175]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.26 (d, J = 8.7 Hz, 2H, PMB), 6.90 (d, J = 8.7 Hz, 2H, PMB), 4.69 – 4.64 (m, 1H, H-5), 4.55 – 4.44 (m, 2H, PMB), 3.82 (s, 3H, PMB), 3.66 (dd, J = 10.8, 3.4 Hz, 1H, H-6a), 3.57 (dd, J = 10.7, 4.2 Hz, 1H, H-6b), 2.67 – 2.58 (m, 1H, H-3a), 2.53 – 2.44 (m, 1H, H-3b), 2.34 – 2.24 (m, 1H, H-4a), 2.17 – 2.06 (m, 1H, H-4b); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 177.5 (s, C-2), 159.5 (s, PMB), 129.4 (d, PMB), 114.0 (d, PMB), 79.2 (d, C-5), 73.4 (t, PMB), 71.3 (t, C-6), 55.4 (q, PMB), 28.6 (t, C-3), 24.3 (t, C-4); **HRMS-ESI** (m/z): $[M+Na]^+$ calcd for $[C_{13}H_{16}O_4Na]^+$: 259.0946, found: 259.0945; **$[\alpha]^{20}_D$** = −9.5° (c = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −9.8°, c = 0.43, $CHCl_3$).[175]

(*R*)-5-(4-Methoxybenzyloxy)pentan-1,4-diol (175b)



$C_{13}H_{20}O_4$
Mol. Wt.: 240.30 g/mol

175b

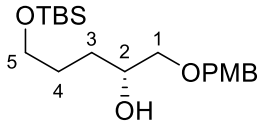
The lactone **174b** (1.05 g, 4.44 mmol, 1.0 eq.) was dissolved in THF (100 mL) under nitrogen atmosphere, cooled down to 0 °C and treated with $LiAlH_4$ portionwise (0.19 g, 4.89 mmol, 1.1 eq.). After 30 min the reaction was terminated by addition of water. After further 10 min the precipitate was filtered through Celite^{TM}, the filtrate was diluted with water and extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 1 : 1 to 1 : 5) to give the diol **175b** (0.78 g, 3.2 mmol, 73%) as a colorless oil.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.29 (d, J = 8.5 Hz, 2H, PMB), 6.91 (d, J = 8.7 Hz, 2H, PMB), 4.51 (s, 2H, PMB), 3.91 – 3.85 (m, 1H, H-4), 3.84 (s, 3H, PMB), 3.74 – 3.63 (m, 2H, H-1), 3.50 (dd, J = 9.4, 3.3 Hz, 1H, H-5a), 3.35 (dd, J = 9.4, 8.0 Hz, 1H, H-5b), 2.84 (bs, 1H, 4-OH), 2.42 (bs, 1H, 1-OH), 1.76 – 1.67 (m, 2H, H-2), 1.66– 1.58 (m, 1H, H-3a), 1.56– 1.47 (m, 1H, H-3b); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 159.5 (s, PMB), 129.6 (d, PMB), 114.0 (d, PMB), 74.3 (d, C-4), 73.2 (t, PMB), 70.6 (t, C-5), 63.0 (t, C-1), 55.4 (q, PMB), 30.3 (t, C-2), 29.3 (t, C-3); **HRMS-ESI** (m/z):

[M+Na]$^+$ calcd for [$C_{13}H_{20}O_4Na$]$^+$: 263.1259, found: 263.1258; **[α]$^{20}_D$** = −2.0° (*c* = 1.0, $CHCl_3$; Lit. **[**α]$^{20}_D$ = −2.0°, *c* = 1.0, $CHCl_3$).[135]

(*R*)-5-(*tert*-Butyldimethylsilyloxy)-1-(4-methoxybenzyloxy)pentan-2-ol (176b)



$C_{19}H_{34}O_4Si$
Mol. Wt.: 354.56 g/mol

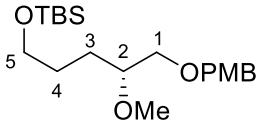
176b

The diol **175b** (0.50 g, 2.08 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (20 mL) under nitrogen atmosphere, cooled down to 0 °C and treated with imidazole (0.28 g, 4.16 mmol, 2.0 eq.) followed by TBSCl (0.35 g, 2.29 mmol, 1.1 eq.). The reaction mixture was warmed up to RT and stirred for 1 h. The reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 5: 1 to 2: 1) to give the protected alcohol **176b** (0.68 g, 1.9 mmol, 92%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.30 (d, *J* = 8.7 Hz, 2H, PMB), 6.92 (d, *J* = 8.7 Hz, 2H, PMB), 4.52 (s, 2H, PMB), 3.94 – 3.84 (m, 1H, H-2), 3.84 (s, 3H, PMB), 3.73 – 3.63 (m, 2H, H-5), 3.51 (dd, *J* = 9.4, 3.7 Hz, 1H, H-1a), 3.37 (dd, *J* = 9.4, 7.4 Hz, 1H, H-1b), 2.92 (bs, 1H, 2-OH), 1.77 – 1.48 (m, 2H, H-4), 1.77 – 1.48 (m, 2H, H-4), 0.93 (s, 9H, Si*t*-Bu), 0.09 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 159.4 (s, PMB), 130.3 (s, PMB), 113.9 (d, PMB), 74.4 (d, C-2), 73.1 (t, PMB), 70.3 (t, C-1), 63.4 (t, C-5), 55.4 (q, PMB), 30.4 (t, C-4), 29.1 (t, C-3), 26.1 (q, Si*t*-Bu), 18.4 (s, Si*t*-Bu), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): [M+Na]$^+$ calcd for [$C_{19}H_{34}O_4NaSi$]$^+$: 377.2124, found: 377.2124; **[α]$^{20}_D$** = +2.5° (*c* = 1.0, $CHCl_3$; Lit. **[**α]$^{20}_D$ = +2.4°, *c* = 1.0, $CHCl_3$).[135]

(*R*)-5-(*tert*-Butyldimethylsilyloxy)-2-methoxy-1-(4-methoxybenzyloxy)pentane (177b)



$C_{20}H_{36}O_4Si$
Mol. Wt.: 368.59 g/mol

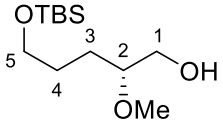
177b

The alcohol **176b** (0.56 g, 1.58 mmol, 1.0 eq.) was dissolved in THF (20 mL) under nitrogen atmosphere, cooled down to 0 °C and treated with NaH (0.06 g, 2.37 mmol, 1.5 eq.) followed by MeI (0.1 mL, 1.74 mmol, 1.1 eq.). The reaction mixture was warmed up to RT and stirred for 24 h. The reaction was terminated by addition of water. The phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 5 : 1 to 2 : 1) to give the protected alcohol **177b** (0.51 g, 1.38 mmol, 88%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.26 (d, J = 8.6 Hz, 2H, PMB), 6.87 (d, J = 8.7 Hz, 2H, PMB), 4.48 (s, 2H, PMB), 3.80 (s, 3H, PMB), 3.63 – 3.57 (m, 2H, H-5), 3.45 (d, J = 4.9 Hz, 2H, H-1), 3.40 (s, 3H, Me), 3.39 – 3.34 (m, 1H, H-2), 1.60 – 1.49 (m, 2H, H-4), 0.89 (s, 9H, Si*t*-Bu), 0.04 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 159.3 (s, PMB), 130.6 (s, PMB), 129.4 (d, PMB), 113.9 (d, PMB), 80.1 (d, C-2), 73.1 (t, PMB), 71.9 (t, C-1), 63.4 (t, C-5), 57.6 (q, OMe), 55.4 (q, PMB), 28.8 (t, C-4), 27.9 (t, C-3), 26.1 (q, Si*t*-Bu), 18.5 (s, Si*t*-Bu), -5.1 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{20}H_{36}O_4NaSi]^+$: 391.2281, found: 391.2283; **$[\alpha]^{20}_D$** = +6.1° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = +6.3°, *c* = 1.0, $CHCl_3$).[135]

(*R*)-5-(*tert*-Butyldimethylsilyloxy)-2-methoxypentan-1-ol (178b)



$C_{12}H_{28}O_3Si$
Mol. Wt.: 248.44 g/mol

178b

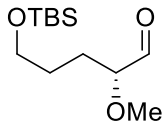
The alcohol **177b** (0.4 g, 1.09 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (30 mL), cooled down to 0 °C and treated with water (3 mL) and DDQ (0.30 g, 1.30

mmol, 1.2 eq.). The reaction mixture was warmed up to RT and stirred for 1.5 h. The reaction was terminated by addition of an aqueous $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and removed under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 15: 1 to 5: 1) and the deprotected alcohol **178b** (0.24 g, 0.97 mmol, 89%) was obtained as a colorless oil.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 3.71 – 3.65 (m, 1H, H-1a), 3.63 – 3.60 (m, 2H, H-5), 3.52 – 3.45 (m, 1H, H-1b), 3.40 (s, 3H, OMe), 3.32 – 3.26 (m, 1H, H-2), 2.08 – 2.05 (m, 1H, OH), 1.66 – 1.47 (m, 2H, H-4), 0.88 (s, 9H, Si*t*-Bu), 0.04 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 81.5 (d, C-2), 64.1 (t, C-1), 63.2 (t, C-5), 57.2 (q, OMe), 28.6 (t, C-4), 26.7 (t, C-3), 26.1 (q, Si*t*-Bu), 18.5 (s, Si*t*-Bu), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{12}H_{28}O_3NaSi]^+$: 271.1705, found: 271.1706; **$[\alpha]^{20}_D$** = −20.6° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −20.8°, *c* = 1.0, $CHCl_3$).[135]

(*R*)-5-(*tert*-Butyldimethylsilyloxy)-2-methoxypentan-1-al (157b)

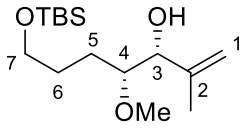


$C_{12}H_{26}O_3Si$
Mol. Wt.: 246.42 g/mol

157b

The alcohol **178b** (500 mg, 2.0 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (100 mL), cooled down to 0 °C and treated with $NaHCO_3$ (203 mg, 2.42 mmol, 1.2 eq.) and DMP reagent (1.0 g, 2.42 mmol, 1.2 eq.). After stirring at RT for 1 h the reaction was terminated by addition of an aqueous Na_2SO_3 solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure providing the aldehyde **157b** (491 mg, 1.99 mmol, 99%) as a colorless solid. The product was used for the next step without further characterization.

(3*R*,4*R*)-7-(*tert*-Butyldimethylsilyloxy)-4-methoxy-2-methylhept-1-en-3-ol (150b)



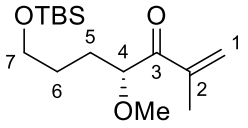
$C_{15}H_{32}O_3Si$
Mol. Wt.: 288.50 g/mol

150b

The aldehyde **157b** (450 mg, 1.83 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in THF (70 mL), cooled down to −78 °C and treated with isopropenylmagnesium bromide (0.5 M in THF, 7.3 mL, 3.65 mmol, 2.0 eq.). After 1 h the reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 5 : 1 to 2 : 1) providing the product **150b** (259 mg, 0.9 mmol, 50%, d.r. *syn* : *anti* = 7 : 1) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 5.02 – 5.00 (m, 1H, H-1a), 4.93 – 4.90 (m, 1H, H-1b), 3.93 (d, J = 6.8 Hz, 1H, H-3), 3.61 – 3.57 (m, 2H, H-7), 3.41 (s, 3H, OMe), 3.27 – 3.20 (m, 1H, H-4), 2.68 (d, 1H, 3-OH), 1.74 (s, 3H, 2-Me), 1.62 – 1.44 (m, 2H, H-5), 1.62 – 1.44 (m, 2H, H-6), 0.88 (s, 9H, Si*t*-Bu), 0.03 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 144.4 (s, C-2), 114.0 (t, C-1), 82.1 (d, C-4), 77.4 (d, C-3), 63.2 (t, C-7), 58.0 (q, OMe), 28.2 (t, C-6), 26.4 (t, C-5), 26.1 (q, Si*t*-Bu), 18.5 (s, Si*t*-Bu), 18.0 (q, 2-Me), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{33}O_3Si]^+$: 289.2199, found: 289.2203; $[\alpha]^{20}_D$ = −30.1° (*c* = 1.25, CH_2Cl_2).

(*R*)-7-(*tert*-Butyldimethylsilyloxy)-4-methoxy-2-methylhept-1-en-3-one (183)



$C_{15}H_{30}O_3Si$
Mol. Wt.: 286.49 g/mol

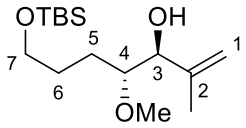
183

The alcohol **150b** (220 mg, 0.76 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (40 mL), cooled down to 0 °C and treated with $NaHCO_3$ (77 mg, 0.92 mmol, 1.2 eq.) and DMP reagent (388 mg, 0.92 mmol, 1.2 eq.). After stirring for 1 h at RT the reaction was terminated by addition of an aqueous Na_2SO_3 solution. The phases were separated and the

aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure providing the ketone **183** (188 mg, 0.66 mmol, 86%) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 6.10 (s, 1H, H-1a), 5.83 (s, 1H, H-1b), 4.32 (dd, *J* = 8.1, 4.5 Hz, 1H, H-4), 3.65 – 3.51 (m, 2H, H-7), 3.29 (s, 3H, OMe), 1.88 (s, 3H, 2-Me), 1.84 – 1.54 (m, 2H, H-5), 1.84 – 1.54 (m, 2H, H-6), 0.86 (s, 9H, Si*t*-Bu), 0.02 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 202.2 (s, C-3), 143.0 (s, C-2), 125.7 (t, C-1), 83.2 (d, C-4), 62.6 (t, C-7), 57.7 (q, OMe), 30.0 (t, C-6), 28.8 (t, C-5), 26.0 (q, Si*t*-Bu), 18.4 (s, Si*t*-Bu), 18.2 (q, 2-Me), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{15}H_{30}O_3NaSi]^+$: 309.1862, found: 309.1862.

(3*S*,4*R*)-7-(*tert*-Butyldimethylsilyloxy)-4-methoxy-2-methylhept-1-en-3-ol (150a)



$C_{15}H_{32}O_3Si$
Mol. Wt.: 288.50 g/mol

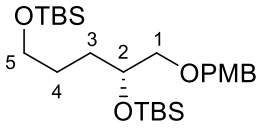
150a

The ketone **183** (144 mg, 0.5 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in THF (70 mL), cooled down to −78 °C and treated with (*S*)-CBS (0.83 g, 3.0 mmol, 6.0 eq.). After 10 min $BH_3{\cdot}SMe_2$ (2.0 M in THF) (6.3 mL, 12.6 mmol, 25.0 eq.) was added and the reaction mixture was stirred at −78 °C for 24 h. The reaction was terminated by addition of MeOH, the mixture was warmed up to RT and diluted with water. The phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product (a mixture of diastereomers 1 : 1) was purified by flash chromatography (PE : EA = 10 : 1 to 5 : 1) providing the desired *anti*-diastereomer **150a** (32 mg, 0.11 mmol, 22%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 5.08 (s, 1H, H-1a), 4.93 – 4.92 (m, 1H, H-1b), 4.28 (d, *J* = 3.8 Hz, 1H, H-3), 3.66 – 3.54 (m, 2H, H-7), 3.41 (s, 3H, OMe), 3.30 – 3.23 (m, 1H, H-4), 2.23 (s, 1H, 3-OH), 1.72 (s, 3H, 2-Me), 1.56 – 1.45 (m, 2H, H-5),), 1.56 – 1.45 (m, 2H, H-6), 0.88 (s, 9H, Si*t*-Bu),0.04 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 143.7 (s, C-2), 111.9 (t, C-1), 82.6 (d, C-4), 74.1 (d, C-3), 63.3 (t, C-7), 57.6 (q, OMe), 29.2 (t, C-6), 26.1 (q, Si*t*-Bu), 24.5 (t, C-5), 19.5 (q, 2-Me), 18.5 (s,

Si*t*-Bu), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{15}H_{33}O_3Si]^+$: 289.2199, found: 289.2198; $[\alpha]^{20}_D = -11.0°$ (*c* = 1.0, CH_2Cl_2).

(*R*)-2,5-Bis(*tert*-butyldimethylsilyloxy)-1-(4-methoxybenzyloxy)pentane (185)



$C_{25}H_{48}O_4Si_2$
Mol. Wt.: 468.82 g/mol

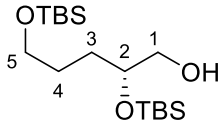
185

The alcohol **175b** (2.50 g, 10.4 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (60 mL), cooled down to 0 °C and treated with 2,6-lutidine (4.20 mL, 36.3 mmol, 3.5 eq.) followed by TBSOTf (5.30 mL, 22.9 mmol, 2.2 eq.). The reaction mixture was warmed up to RT and stirred at RT for additional 2.5 h. The reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 50 : 1 to 10: 1) to give the protected alcohol **185** (4.0 g, 8.53 mmol, 82%) as a colorless foam.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.26 (d, *J* = 8.9 Hz, 2H, PMB), 6.87 (d, *J* = 8.7 Hz, 2H, PMB), 4.45 (s, 2H, PMB), 3.90 – 3.81 (m, 1H, H-2), 3.81 (s, 3H, PMB), 3.62 – 3.59 (m, 2H, H-5), 3.42 – 3.32 (m, 2H, H-1), 1.63 – 1.43 (m, 2H, H-4), 1.63 – 1.43 (m, 2H, H-3), 0.89 (s, 9H, Si*t*-Bu), 0.88 (s, 9H, Si*t*-Bu), 0.05 (s, 6H, SiMe), 0.04 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 159.2 (s, PMB), 130.8 (s, PMB), 129.3 (d, PMB), 113.8 (d, PMB), 74.6 (d, C-1), 73.1 (t, PMB), 71.5 (t, C-2), 63.5 (t, C-5), 55.4 (q, PMB), 31.2 (t, C-3), 28.8 (t, C-4), 26.1 (q, Si*t*-Bu), 18.5 (s, Si*t*-Bu), 18.3 (s, Si*t*-Bu), -4.2 (q, SiMe), -4.6 (q, SiMe), -5.1 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{25}H_{48}O_4NaSi_2]^+$: 491.2989, found: 491.2986; $[\alpha]^{20}_D$ = +10.0° (*c* = 1.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = +10.3°, *c* = 1.0, $CHCl_3$).[135]

(*R*)-2,5-Bis(*tert*-butyldimethylsilyloxy)pentan-1-ol (186)



$C_{17}H_{40}O_3Si_2$
Mol. Wt.: 348.67 g/mol

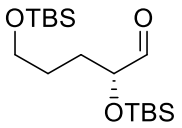
186

The alcohol **185** (2.0 g, 4.27 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (130 mL, cooled down to 0 °C and treated with water (13 mL) and DDQ (1.16 g, 5.12 mmol, 1.2 eq.). The reaction mixture was warmed up to RT and stirred for 1.5 h. The reaction was terminated by addition of an aqueous $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with aqueous NaCl solution, dried over $MgSO_4$ and removed under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 15 : 1 to 5 : 1) and the deprotected alcohol **186** (1.2 g, 3.4 mmol, 80%) was obtained as a colorless oil.

The analytical data are in accordance with the reported in the literature.[135,151]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 3.79 – 3.74 (m, 1H, H-2), 3.63 – 3.54 (m, 2H, H-5), 3.63 – 3.54 (m, 1H, H-1a), 3.48 – 3.43 (m, 1H, H-1b), 1.92 – 1.89 (m, 1H, OH), 1.57 – 1.47 (m, 2H, H-3), 1.57 – 1.47 (m, 2H, H-4), 0.90 (s, 9H, Si*t*-Bu), 0.89 (s, 9H, Si*t*-Bu), 0.09 (s, 6H, SiMe), 0.04 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 72.9 (d, C-2), 66.4 (t, C-1), 63.3 (t, C-5), 30.5 (t, C-3), 28.7 (t, C-4), 26.1 (q, Si*t*-Bu), 18.5 (s, Si*t*-Bu), 18.3 (s, Si*t*-Bu),), -4.3 (q, SiMe), -4.4 (q, SiMe), -5.2 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{17}H_{40}O_3NaSi_2]^+$: 371.2414, found: 371.2413; $\mathbf{[\alpha]^{20}_D}$ = −13.0° (*c* = 2.0, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −13.3°, *c* = 2.0, $CHCl_3$).[135]

(*R*)-2,5-Bis(*tert*-butyldimethylsilyloxy)pentan-1-al (157c)



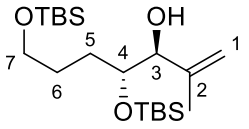
$C_{17}H_{38}O_3Si_2$
Mol. Wt.: 346.66 g/mol

157c

The alcohol **186** (50.0 mg, 0.14 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (10 mL), cooled down to 0 °C and treated with $NaHCO_3$ (14.5 mg, 0.172 mmol, 1.2 eq.) and DMP reagent (73 mg, 0.172 mmol, 1.2 eq.). After stirring at RT for 1 h the reaction

was terminated by addition of an aqueous Na_2SO_3 solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure providing the aldehyde **157c** (49 mg, 0.14 mmol, 99%) as a colorless solid. The product was used for the next step without further characterization.

(3*S*,4*R*)-4,7-Bis(*tert*-butyldimethylsilyloxy)-2-methylhept-1-en-3-ol (151)



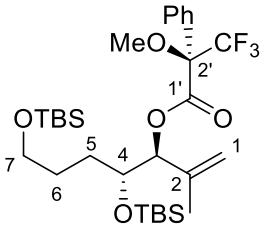
$C_{20}H_{44}O_3Si_2$
Mol. Wt.: 388.74 g/mol

151

The aldehyde **157c** (49 mg, 0.14 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in Et_2O (15 mL), cooled down to −78 °C and treated with isopropenylmagnesium bromide (0.5 M in THF, 0.35 mL, 0.17 mmol, 1.2 eq.). After 30 min the reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 20 : 1) providing the product **151** (27.7 mg, 0.071 mmol, 50%, d.r. *anti* : *syn* = 8 : 1) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 5.07 (s, 1H, H-1a), 4.90 (s, 1H, H-1b), 4.09 (d, J = 3.1 Hz, 1H, H-3), 3.84 – 3.78 (m, 1H, H-4), 3.63 – 3.55 (m, 2H, H-7), 2.51 (s, 1H, 3-OH), 1.71 (s, 3H, 2-Me), 1.66 – 1.42 (m, 2H, H-5), 1.66 – 1.42 (m, 2H, H-6), 0.91 (s, 9H, Si*t*-Bu), 0.88 (s, 9H, Si*t*-Bu), 0.1 (s, 6H, SiMe), 0.03 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 143.1 (s, C-2), 111.8 (t, C-1), 77.6 (d, C-3), 74.0 (d, C-4), 66.4 (t, C-7), 29.3 (t, C-6), 26.9 (t, C-5), 26.1 (q, Si*t*-Bu), 26.0 (q, Si*t*-Bu), 19.6 (q, 2-Me), 18.5 (s, Si*t*-Bu), 18.2 (s, Si*t*-Bu), -4.3 (q, SiMe), -4.4 (q, SiMe), -5.1 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{20}H_{45}O_3Si_2]^+$: 389.2907, found: 389.2901.

(*R*)-Mosher ester (187a)



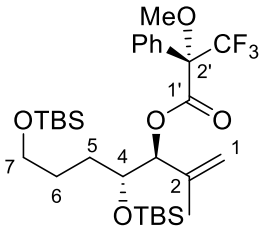
$C_{30}H_{51}F_3O_5Si_2$
Mol. Wt.: 604.90 g/mol

(*R*)-**187a**

The alcohol **151** (13 mg, 33.4 µmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (1.5 mL), cooled down to 0 °C and treated with Et_3N (18.6 µL, 133.8 µmol, 4.0 eq.) and DMAP (0.4 mg, 3.34 µmol, 0.1 eq.) followed by (*S*)-Mosher acid chloride (10.1 mg, 40.1 µmol, 1.2 eq.). The reaction mixture was warmed up to RT and stirred for 3 h. The reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 20 : 1) to give the ester (*R*)-**187a** (16 mg, 26.5 µmol, 79%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.58 – 7.50 (m, 2H, Ph), 7.39 – 7.37 (m, 3H, H-Ph), 5.38 (d, J = 4.7 Hz, 1H, H-3), 5.02 (s, 1H, H-1a), 5.00 (s, 1H, H-1b), 3.91 – 3.80 (m, 1H, H-4), 3.64 – 3.57 (m, 1H, H-7a), 3.54 (s, 3H, 2'-OMe), 3.48 – 3.43 (m, 1H, H-7b), 1.78 (s, 3H, 2-Me), 1.57 – 1.45 (m, 2H, H-5), 1.57 – 1.45 (m, 2H, H-6), 0.87 (s, 9H, Si*t*-Bu), 0.85 (s, 9H, Si*t*-Bu), 0.04 (s, 6H, SiMe), 0.01 (s, 6H, SiMe); **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{30}H_{52}O_5F_3Si_2]^+$: 605.3305, found: 605.3307.

(*S*)-Mosher ester (187b)



$C_{30}H_{51}F_3O_5Si_2$
Mol. Wt.: 604.90 g/mol

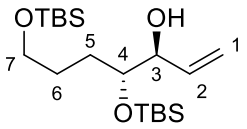
(*S*)-**187b**

The alcohol **151** (12.5 mg, 32.2 µmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (1.5 mL), cooled down to 0 °C and treated with Et_3N (18 µl, 128.6 µmol, 4.0 eq.) and

DMAP (0.4 mg, 3.21 µmol, 0.1 eq.) followed by (*R*)-Mosher acid chloride (9.75 mg, 38.6 µmol, 1.2 eq.). The reaction mixture was warmed up to RT and stirred for 3 h. The reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 20 : 1) to give the ester (*S*)-**187b** (13.1 mg, 21.7 µmol, 67%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.64 – 7.57 (m, 2H, Ph), 7.39 – 7.34 (m, 3H, H-Ph), 5.45 (d, *J* = 3.1 Hz, 1H, H-3), 4.86 (s, 1H, H-1a), 4.74 (s, 1H, H-1b), 3.91 – 3.88 (m, 1H, H-4), 3.61 (s, 3H, 2'-OMe), 3.58 – 3.52 (m, 2H, H-7), 1.69 (s, 3H, 2-Me), 1.65 – 1.43 (m, 2H, H-5), 1.65 – 1.43 (m, 2H, H-6), 0.87 (s, 9H, Si*t*-Bu), 0.86 (s, 9H, Si*t*-Bu), 0.09 (s, 6H, SiMe), 0.01 (s, 6H, SiMe); **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{30}H_{52}O_5F_3Si_2]^+$: 605.3305, found: 605.3312.

(3*S*,4*R*)-4,7-Bis(*tert*-butyldimethylsilyloxy)-2-hept-1-en-3-ol (152)



$C_{19}H_{42}O_3Si_2$
Mol. Wt.: 374.71 g/mol

152

The aldehyde **157c** (0.98 g, 2.82 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in Et_2O (200 mL), cooled down to −90 °C and treated with vinylmagnesium bromide (0.7 M in THF) (5 mL, 3.4 mmol, 1.2 eq.). After 30 min the reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 20 : 1) providing the product **152** (0.64 g, 1.7 mmol, 60%, d.r. *anti* : *syn* = 7 : 1) as a colorless oil.

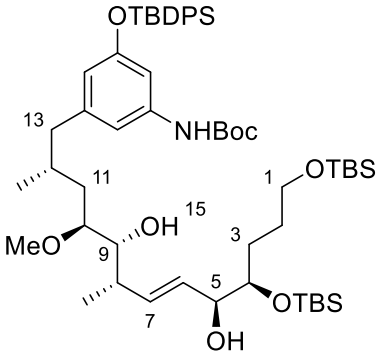
The analytical data are in accordance with the reported in the literature.[151]

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 5.90 – 5.80 (m, 1H, H-2), 5.29 (dd, *J* = 17.3, 1.5 Hz, 1H, H-1a), 5.19 (dd, *J* = 10.5, 1.4 Hz, 1H, H-1b), 4.09 – 4.13 (m, 1H, H-3), 3.74 – 3.70 (m, 1H, H-4), 3.64 – 3.55 (m, 2H, H-7), 2.27 (d, *J* = 2.8 Hz, 1H, 3-OH), 1.66 –

1.45 (m, 2H, H-5), 1.66 – 1.45 (m, 2H, H-6), 0.90 (s, 9H, Si*t*-Bu), 0.88 (s, 9H, Si*t*-Bu), 0.09 (s, 6H, SiMe), 0.04 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 136.5 (d, C-2), 116.5 (t, C-1), 75.8 (d, C-3), 75.2 (d, C-4), 63.2 (t, C-7), 28.9 (t, C-6), 28.0 (t, C-5), 26.0 (q, Si*t*-Bu), 25.9 (q, Si*t*-Bu), 18.3 (s, Si*t*-Bu), 18.1 (s, Si*t*-Bu), -4.4 (q, SiMe), -5.3 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{19}H_{42}O_3Si_2Na]^+$: 397.2570, found: 397.2570.

3.7 Synthesis of Progeldanamycin Derivatives

Metathesis product 195



$C_{55}H_{91}NO_8Si_3$
Mol. Wt.: 978.59 g/mol

195

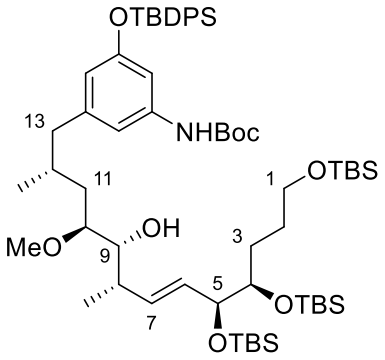
The western fragment **148** (63 mg, 0.1 mmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (3 mL) and treated with the eastern fragment **152** (112 mg, 0.3 mmol, 3.0 eq.) and Hoveyda-Grubbs II catalyst (6.2 mg, 0.01 mmol, 0.1 eq.). The reaction mixture was stirred at 40 °C for 48 h. After 10 h an additional portion of the catalyst (6.2 mg, 0.01 mmol, 0.1 eq.) was added. After 48 h the mixture was cooled down to RT and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 10 : 1 to 4 :1) and preparative HPLC (solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/20; 5/20; 80/100; 100/100; t_R = 90.0 min) providing the product **195** (39 mg, 39.8 µmol, 40%) as a colorless oil.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, C_6D_6, C_6D_5H = 7.16 ppm) δ (ppm): 7.90 – 7.84 (m, 4H, SiPh), 7.23 – 7.17 (m, 6H, SiPh), 7.10 (s, 1H, H-17), 6.78 (m, 1H, H-15), 6. 48 (s, 1H, H-19), 5.92 (bs, 1H, NH), 5.70 (dd, *J* = 15.6, 8.2 Hz, 1H, H-7), 5.60 (dd, *J* = 15.6, 6.4 Hz, 1H, H-6), 4.14 (bs, 1H,

H-5), 3.76 – 3.69 (m, 1H, H-4), 3.62 – 3.56 (m, 1H, H-9), 3.62 – 3.56 (m, 1H, H-1), 3.27 – 3.23 (m, 1H, H-10), 3.04 (s, 3H, 10-OMe), 2.40 (dd, *J* = 13.3, 5.9 Hz, 1H, H-13a), 2.44 – 2.39 (m, 1H, H-8), 2.12 (dd, *J* = 13.1, 8.4 Hz, 1H, H-13b), 1.96 (bs, 1H, 9-OH), 1.99 – 1.90 (m, 1H, H-12), 1.78 – 1.68 (m, 1H, H-2a), 1.78 – 1.68 (m, 1H, H-3a), 1.78 – 1.68 (m, 1H, H-11a), 1.63 – 1.56 (m, 1H, H-2b), 1.63 – 1.56 (m, 1H, H-3b), 1.38 (s, 9H, *t*-Bu), 1.32 (d, *J* = 6.6 Hz, 3H, 8-Me), 1.20 (s, 9H, Si*t*-Bu), 1.14 – 1.10 (m, 1H, H-11b), 1.00 (s, 9H, Si*t*-Bu), 0.97 (s, 9H, Si*t*-Bu), 0.77 (d, *J* = 6.5 Hz, 3H, 12-Me), 0.11 (s, 6H, SiMe), 0.09 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, C_6D_6, C_6D_6 = 128.06 ppm) δ (ppm): 156.4 (s, C-Ar), 152.4 (s, NHCOO), 143.4 (s, C-Ar), 140.1 (d, C-Ar), 136.0 (s, C-Ar), 134.2 (d, C-7), 133.6 (d, C-Ar), 130.1 (s, C-Ar), 129.4 (d, C-6), 115.7 (d, C-19), 112.6 (d, C-15), 107.9 (d, C-17), 80.5 (d, C-10), 79.6 (s, *t*-Bu), 76.1 (d, C-4), 75.4 (d, C-5), 73.8 (d, C-9), 63.5 (t, C-1), 56.7 (q, 10-OMe), 45.0 (t, C-13), 39.3 (d, C-8), 35.3 (t, C-11), 31.4 (d, C-12), 29.4 (t, C-2), 29.0 (t, C-3), 28.3 (q, *t*-Bu), 26.8 (q, Si*t*-Bu), 26.2 (q, Si*t*-Bu), 19.8 (s, Si*t*-Bu), 19.2 (q, 12-Me), 18.6 (s, Si*t*-Bu), 18.4 (s, Si*t*-Bu), 17.5 (q, 8-Me), -4.2 (q, SiMe), -5.1 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{55}H_{91}NO_8Si_3Na]^+$: 1000.5950, found: 1000.5946; **$[\alpha]^{20}_D$** = −20.0° (*c* = 0.5, $CHCl_3$; Lit. $[\alpha]^{20}_D$ = −18.2°, *c* = 0.33, $CHCl_3$).[135]

Silylated polyol 211



$C_{67}H_{105}NO_8Si_4$
Mol. Wt.: 1092.85 g/mol

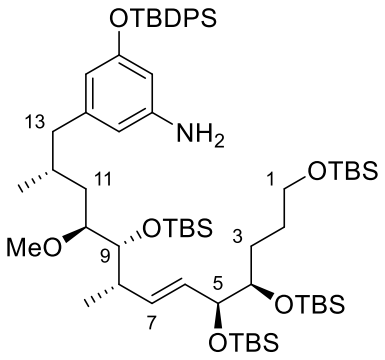
211

The metathesis product **195** (50 mg, 51.1 µmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (10 mL), cooled down to 0 °C and treated with 2,6-lutidine (30 µL, 256 µmol, 5.0 eq.) and TBSOTf (35 µL, 153.3 µmol, 3.0 eq.). The reaction mixture was warmed up to RT and stirred for 3 h. The reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by

flash chromatography (PE : EA = 10 : 1 to 5 : 1) providing the product **211** (40 mg, 36.3 μmol, 71%) as a colorless oil.

^{1}H NMR (400 MHz, C_6D_6, C_6D_5H = 7.16 ppm) δ (ppm): 7.88 – 7.82 (m, 4H, SiPh), 7.24 – 7.18 (m, 6H, SiPh), 7.05 (s, 1H, H-17), 6.81 (s, 1H, H-15), 6. 50 (s, 1H, H-19), 5.87 (bs, 1H, NH), 5.65 (dd, *J* = 15.6, 6.8 Hz, 1H, H-6), 5.46 (dd, *J* = 15.6, 8.2 Hz, 1H, H-7), 4.11 – 4.09 (m, 1H, H-5), 3.81 – 3.73 (m, 1H, H-4), 3.66 – 3.63 (m, 2H, H-1), 3.60 – 3.57 (m, 1H, H-9), 3.30 – 3.27 (m, 1H, H-10), 3.13 (s, 3H, 10-OMe), 2.43 (dd, *J* = 13.1, 6.5 Hz, 1H, H-13a), 2.38 – 2.32 (m, 1H, H-8), 2.16 (dd, *J* = 13.1, 8.7 Hz, 1H, H-13b), 2.05 – 1.99 (m, 1H, H-12), 1.97 (bs, 1H, 9-OH), 1.82 – 1.72 (m, 2H, H-2), 1.71 – 1.67 (m, 2H, H-3), 1.65 – 1.63 (m, 1H, H-11a), 1.38 (s, 9H, *t*-Bu), 1.33 (d, *J* = 6.6 Hz, 3H, 8-Me), 1.20 (s, 9H, Si*t*-Bu), 1.14 – 1.09 (m, 1H, H-11b), 1.05 (s, 9H, Si*t*-Bu), 1.04 (s, 9H, Si*t*-Bu), 1.00 (s, 9H, Si*t*-Bu), 0.79 (d, *J* = 6.5 Hz, 3H, 12-Me), 0.21 (s, 3H, SiMe), 0.17 (s, 3H, SiMe), 0.16 (s, 3H, SiMe), 0.14 (s, 3H, SiMe), 0.10 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, C_6D_6, C_6D_6 = 128.06 ppm) δ (ppm): 156.4 (s, C-Ar), 152.3 (s, NHCOO), 143.4 (s, C-Ar), 140.1 (d, C-Ar), 136.0 (s, C-Ar), 134.2 (d, C-7), 133.6 (d, C-Ar), 131.0 (s, C-6), 130.1 (d, C-Ar), 115.7 (d, C-19), 112.6 (d, C-15), 108.0 (d, C-17), 80.6 (d, C-10), 79.6 (s, *t*-Bu), 78.2 (d, C-5), 77.2 (d, C-4), 73.6 (d, C-9), 63.5 (t, C-1), 56.8 (q, 10-OMe), 45.0 (t, C-13), 39.2 (d, C-8), 35.1 (t, C-11), 31.3 (d, C-12), 30.3 (t, C-3), 29.5 (t, C-2), 28.3 (q, *t*-Bu), 26.8 (q, Si*t*-Bu), 26.4 (q, Si*t*-Bu), 26.2 (q, Si*t*-Bu), 25.9 (q, Si*t*-Bu), 19.8 (s, Si*t*-Bu), 19.2 (q, 12-Me), 18.7 (s, Si*t*-Bu), 18.6 (s, Si*t*-Bu), 18.6 (q, 8-Me), 17.4 (s, Si*t*-Bu), -3.3 (q, SiMe), -3.6 (q, SiMe), -3.7 (q, SiMe), -4.2 (q, SiMe), -4.3 (q, SiMe), -5.1 (q, SiMe); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{61}H_{105}NO_8Si_4Na]^+$: 1114.6815, found: 1114.6814.

Boc-deprotected polyol 212



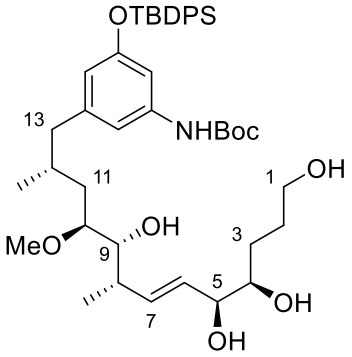
$C_{62}H_{111}NO_6Si_5$
Mol. Wt.: 1107.00 g/mol

212

The metathesis product **195** (50 mg, 51.1 µmol, 1.0 eq.) was dissolved under nitrogen atmosphere in CH_2Cl_2 (10 mL), cooled down to 0 °C and treated with 2,6-lutidine (30 µL, 256 µmol, 5.0 eq.) and TBSOTf (35 µL, 153.3 µmol, 3.0 eq.). The reaction mixture was warmed up to RT and stirred for 3 h. After that time an additional portion of TBSOTf (17 µL, 77 µmol, 1.5 eq.) was added at 0 °C and the reaction mixture was stirred at RT for 12 h. The reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 100 : 1 to 50 : 1) providing the product **212** (15 mg, 13.6 µmol, 26%) as a colorless oil.

^{1}H NMR (400 MHz, C_6D_6, C_6D_5H = 7.16 ppm) δ (ppm): 7.97 – 7.94 (m, 4H, SiPh), 7.33 – 7.31 (m, 6H, SiPh), 6.63 (s, 1H, H-17), 6.57 (s, 1H, H-15), 6.15 (s, 1H, H-19), 5.73 (dd, *J* = 15.5, 7.0 Hz, 1H, H-6), 5.55 (dd, *J* = 15.5, 8.8 Hz, 1H, H-7), 4.21 (dd, *J* = 6.9, 2.1 Hz, 1H, H-5), 3.90 – 3.88 (m, 1H, H-4), 3.78 – 3.69 (m, 1H, H-9), 3.78 – 3.69 (m, 2H, H-1), 3.40 – 3.35 (m, 1H, H-10), 3.33 (s, 3H, 10-OMe), 2.49 (dd, *J* = 13.2, 6.6 Hz, 1H, H-13a), 2.36 (dd, *J* = 15.3, 8.4 Hz, 1H, H-8), 2.27 (dd, *J* = 13.6, 5.7 Hz, 1H, H-13b), 2.07 (bs, 1H, 5-OH), 1.98 – 1.93 (m, 1H, H-12), 1.92 – 1.83 (m, 2H, H-2), 1.81 – 1.77 (m, 2H, H-3), 1.74 – 1.71 (m, 1H, H-11a), 1.29 (s, 9H, Si*t*-Bu), 1.20 (d, *J* = 6.0 Hz, 3H, 8-Me), 1.17 (s, 9H, Si*t*-Bu), 1.13 – 1.11 (m, 1H, H-11b), 1.15 (s, 9H, Si*t*-Bu), 1.10 (s, 9H, Si*t*-Bu), 1.06 (s, 9H, Si*t*-Bu), 0.89 (d, *J* = 6.6 Hz, 3H, 12-Me), 0.33 (s, 3H, SiMe), 0.30 (s, 3H, SiMe), 0.29 (s, 3H, SiMe), 0.27 (s, 3H, SiMe), 0.26 (s, 6H, SiMe), 0.19 (s, 6H, SiMe); **^{13}C NMR** (100 MHz, C_6D_6, C_6D_6 = 128.06 ppm) δ (ppm): 156.5 (s, C-Ar), 143.3 (s, C-Ar), 139.8 (d, C-Ar), 136.0 (s, C-Ar), 135.1 (d, C-7), 133.6 (d, C-Ar), 130.7 (s, C-6), 130.1 (d, C-Ar), 116.0 (d, C-19), 113.8 (d, C-15), 110.7 (d, C-17), 81.5 (d, C-10), 78.5 (d, C-5), 77.5 (d, C-4), 76.1 (d, C-9), 63.5 (t, C-1), 57.2 (q, 10-OMe), 45.2 (t, C-13), 41.3 (d, C-8), 35.7 (t, C-11), 31.4 (d, C-12), 30.3 (t, C-3), 29.7 (t, C-2), 26.9 (q, Si*t*-Bu), 26.6 (q, Si*t*-Bu), 26.4 (q, Si*t*-Bu), 26.4 (q, Si*t*-Bu), 26.2 (q, Si*t*-Bu), 19.8 (s, Si*t*-Bu), 19.6 (q, 12-Me), 18.9 (s, Si*t*-Bu), 18.7 (s, Si*t*-Bu), 18.7 (s, Si*t*-Bu), 18.6 (q, 8-Me), 18.4 (s, Si*t*-Bu), -3.4 (q, SiMe), -3.6 (q, SiMe), -3.6 (q, SiMe), -4.1 (q, SiMe), -4.3 (q, SiMe), -4.4 (q, SiMe), -4.5 (q, SiMe), -5.1 (q, SiMe).

TBS-deprotected polyol 197



$C_{43}H_{63}NO_8Si$
Mol. Wt.: 750.06 g/mol

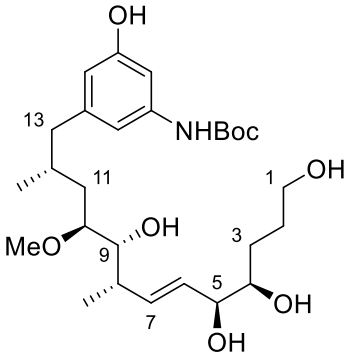
197

The metathesis product **195** (40 mg, 40.9 µmol, 1.0 eq.) was dissolved in MeOH (1 mL) and treated with PPTS (0.61 g, 2.45 mmol, 60.0 eq.). The reaction mixture was heated to 40 °C and stirred for 48 h. The reaction was terminated by addition of an aqueous NH_4Cl solution and MeOH was removed under reduced pressure. The residue was extracted three times with EtOAc, the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by preparative HPLC (solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 15.0 mL/min; gradient (*t* [min]/solvent B [%]): 0/30; 5/30; 50/60; 90/100, 100/100; t_R = 74.0 min) providing the product **197** (28 mg, 36.8 µmol, 90%) as a colorless oil.

^{1}H NMR (400 MHz, CD_3OD, CHD_2OD = 3.31 ppm) δ (ppm): 7.76 – 7.73 (m, 4H, SiPh), 7.48 – 7.39 (m, 6H, SiPh), 6.93 (s, 1H, Ph), 6.73 (s, 1H, Ph), 6.13 (s, 1H, Ph), 5.60 – 5.58 (m, 1H, H-6), 5.60 – 5.58 (m, 1H, H-7), 3.92 – 3.87 (m, 1H, H-5), 3.60 – 3.57 (m, 2H, H-1), 3.55–3.48 (m, 1H, H-9), 3.55 – 3.48 (m, 1H, H-4), 3.37 – 3.35 (m, 1H, H-10), 3.28 (s, 3H, 10-OMe), 2.38 (dd, *J* = 13.2, 5.6 Hz, 1H, H-13a), 2.27 – 2.24 (m, 1H, H-8), 2.08 (dd, *J* = 13.2, 8.8 Hz, 1H, H-13b), 1.79 – 1.73 (m, 1H, H-12), 1.72 – 1.54 (m, 2H, H-2), 1.72 – 1.54 (m, 2H, H-3), 1.51 (s, 9H, *t*-Bu), 1.45 – 1.38 (m, 1H, H-11a), 1.28 – 1.25 (m, 1H, H-11b), 1.11 (d, *J* = 6.5 Hz, 3H, 8-Me), 1.11 (s, 9H, Si*t*-Bu), 0.64 (d, *J* = 6.5 Hz, 3H, 12-Me); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 157.1 (s, C-Ar), 155.2 (s, NHCOO), 143.9 (s, C-Ar), 141.2 (s, C-Ar), 136.7 (d, C-Ar), 136.3 (d, C-7), 134.3 (s, C-Ar), 131.1 (d, C-Ar), 130.8 (d, C-6), 128.9 (d, C-Ar), 116.1 (d, C-Ar), 113.8 (d, C-Ar), 109.2 (d, C-Ar), 81.4 (d, C-10), 80.7 (s, *t*-Bu), 76.9 (d, C-4), 75.6 (d, C-5), 75.2 (d, C-9), 63.1 (t, C-1), 57.1 (q, 10-OMe), 45.7 (t, C-13), 40.7 (d, C-8), 36.6 (t, C-11), 32.3 (d, C-12), 30.1 (t, C-3), 30.1 (t, C-2), 28.7 (q,

t-Bu), 27.1 (q, Si*t*-Bu), 20.2 (s, Si*t*-Bu), 19.3 (q, 12-Me), 17.5 (q, 8-Me); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{43}H_{63}NO_8SiNa]^+$: 772.4221, found: 772.4221.

Polyol 198



$C_{27}H_{45}NO_8$
Mol. Wt.: 511.66 g/mol

198

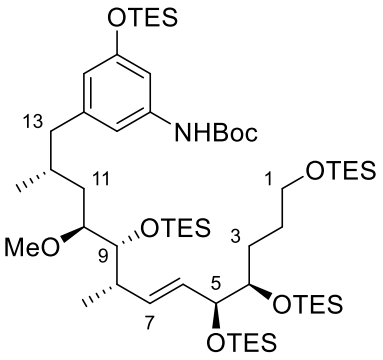
TBS-Deprotected polyol **197** (26 mg, 34.7 µmol, 1.0 eq.) was dissolved in THF (4 mL) under nitrogen atmosphere, cooled down to −78 °C and treated with TBAF·$3H_2O$ (55 mg, 173.3 µmol, 5.0 eq.). The reaction mixture was stirred at −78 °C for 20 h. After that time the reaction was terminated by addition of an aqueous NH_4Cl solution, the phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by semi-preparative HPLC (solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/30; 5/30; 50/60; 90/100, 100/100; t_R = 26.0 min) providing the product **198** (15 mg, 29.3 µmol, 80%) as a colorless oil.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, CD_3OD, CHD_2OD = 3.31 ppm) δ (ppm): 6.82 (s, 1H, Ph), 6.63 (s, 1H, Ph), 6.29 (s, 1H, Ph), 5.64 – 5.53 (m, 1H, H-6), 5.64 – 5.53 (m, 1H, H-7), 3.92 – 3.89 (m, 1H, H-5), 3.58 – 3.55 (m, 2H, H-1), 3.58– 3.55 (m, 1H, H-9), 3.48 – 3.45 (m, 1H, H-4), 3.37 – 3.35 (m, 1H, H-10), 3.33 (s, 3H, 10-OMe), 2.49 (dd, *J* = 13.1, 6.5 Hz, 1H, H-13a), 2.33 (dd, *J* = 13.5, 7.9 Hz, 1H, H-13a), 2.27 – 2.21 (m, 1H, H-8), 2.01 – 1.96 (m, 1H, H-12), 1.73 – 1.61 (m, 2H, H-2), 1.73 – 1.61 (m, 2H, H-3), 1.51 (s, 9H, *t*-Bu), 1.43 – 1.37 (m, 1H, H-11a), 1.25 – 1.19 (m, 1H, H-11b), 1.10 (d, *J* = 6.6 Hz, 3H, 8-Me), 0.87 (d, *J* = 6.5 Hz, 3H, 12-Me); **^{13}C NMR** (100 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 158.6 (s, C-Ar), 155.2 (s, NHCOO), 143.9 (s, C-Ar), 141.3 (s, C-Ar), 136.3 (d, C-7), 130.8 (d, C-6), 112.3 (d, C-Ar), 111.7 (d, C-

Ar), 104.6 (d, C-Ar), 81.4 (d, C-10), 80.7 (s, *t*-Bu), 76.8 (d, C-4), 75.6 (d, C-5), 75.2 (d, C-9), 63.1 (t, C-1), 57.0 (q, 10-OMe), 45.9 (t, C-13), 40.8 (d, C-8), 36.3 (t, C-11), 32.3 (d, C-12), 30.1 (t, C-3), 30.1 (t, C-2), 28.8 (q, *t*-Bu), 19.7 (q, 12-Me), 17.7 (q, 8-Me); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{27}H_{45}NO_8Na]^+$: 534.3043, found: 534.3040.

TES-protected polyol 199



$C_{57}H_{115}NO_8Si_5$
Mol. Wt.: 1082.97 g/mol

199

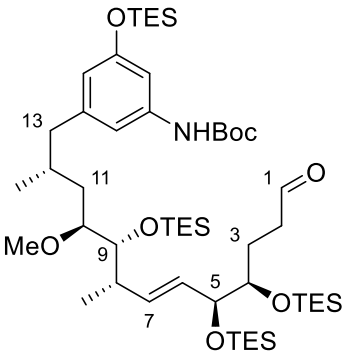
Polyol **198** (5 mg, 9.8 µmol, 1.0 eq.) was dissolved in CH_2Cl_2 (5 mL) under nitrogen atmosphere, cooled down to −78 °C and treated with 2,6-lutidine (9 µL, 78 µmol, 8.0 eq.) and TESOTf (13 µL, 59 µmol, 6.0 eq.). The reaction mixture was stirred at −78 °C for 1 h and warmed up to RT. The reaction was terminated by addition of an aqueous NH_4Cl solution, the phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by purified by flash chromatography (PE : EA = 100 : 1 to 20 : 1) providing the product **199** (8.6 mg, 8.0 µmol, 82%) as a colorless oil.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, C_6D_6, C_6D_5H = 7.16 ppm) δ (ppm): 7.46 (s, 1H, Ph), 6.63 (s, 1H, Ph), 6.38 (s, 1H, Ph), 6.38 (s, 1H, NH), 5.75 (dd, *J* = 15.5, 7.3 Hz, 1H, H-6), 5.75 (dd, *J* = 15.5, 8.9 Hz, 1H, H-7), 4.07 (dd, *J* = 7.2, 1.9 Hz, 1H, H-5), 3.87 – 3.83 (m, 1H, H-4), 3.74 – 3.62 (m, 2H, H-1), 3.74 – 3.62 (m, 1H, H-9), 3.41 (d, *J* = 10.4 Hz, 1H, H-10), 3.30 (s, 3H, 10-OMe), 2.61 (dd, *J* = 13.2, 6.5 Hz, 1H, H-13a), 2.44 – 2.37 (m, 1H, H-13b), 2.36 – 2.32 (m, 1H, H-8), 2.23 – 2.16 (m, 1H, H-12), 2.04 – 1.98 (m, 2H, H-11a), 1.90 – 1.80 (m, 1H, H-2a), 1.72 – 1.65 (m, 2H, H-3), 1.72 – 1.65 (m, 1H, H-2b), 1.43 (s, 9H, *t*-Bu), 1.25 (d, *J* = 6.6 Hz, 3H, 8-Me), 1.22 – 1.18 (m, 1H, H-11b), 1.14 – 1.02 (m, 45H, $SiCH_2C\mathit{H_3}$), 0.92 – 0.97 (m, 3H, 12-Me), 0.82 – 0.61 (m, 30H, $SiC\mathit{H_2}CH_3$); **^{13}C NMR** (100 MHz, C_6D_6, C_6D_6 = 128.06 ppm)

δ (ppm): 156.7 (s, C-Ar), 152.4 (s, NHCOO), 143.4 (s, C-Ar), 140.1 (s, C-Ar), 135.0 (d, C-7), 130.9 (d, C-6), 115.9 (d, C-Ar), 112.2 (d, C-Ar), 107.8 (d, C-Ar), 81.4 (d, C-10), 79.5 (s, *t*-Bu), 78.4 (d, C-5), 77.5 (d, C-4), 76.1 (d, C-9), 63.3 (t, C-1), 57.3 (q, 10-OMe), 45.4 (t, C-13), 41.5 (d, C-8), 35.7 (t, C-11), 31.6 (d, C-12), 30.6 (t, C-2), 30.1 (t, C-3), 28.4 (q, *t*-Bu), 19.7 (q, 12-Me), 18.6 (q, 8-Me), 7.5 (q, $SiCH_2CH_3$), 7.3 (q, $SiCH_2CH_3$), 7.2 (q, $SiCH_2CH_3$), 7.0 (q, $SiCH_2CH_3$), 6.9 (q, $SiCH_2CH_3$), 5.7 (t, $SiCH_2CH_3$), 5.5 (t, $SiCH_2CH_3$), 4.9 (t, $SiCH_2CH_3$); **HRMS-ESI** (*m*/*z*): the compound is not detectable by mass spectrometry.

Silylated aldehyde 200



200

$C_{51}H_{99}NO_8Si_4$
Mol. Wt.: 966.69 g/mol

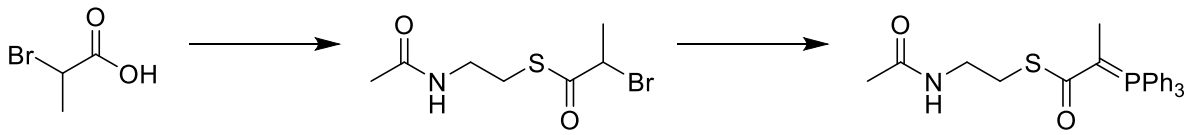
A solution of DMSO (0.9 µL, 12.1 µmol, 2.2 eq.) in CH_2Cl_2 (0.5 mL) was slowly added to a cooled to −78 °C solution of oxalyl chloride (0.5 µL, 6.0 µmol, 1.1 eq.) in CH_2Cl_2 (0.5 mL) After 30 min TES-protected polyol **199** (6.0 mg, 5.5 µmol, 1.0 eq.) dissolved in CH_2Cl_2 (0.5 mL) was slowly added. The reaction mixture was stirred at −78 °C for 1.5 h, followed by addition of DIPEA (5.0 µL, 27.5 µmol, 5.0 eq). The mixture was allowed to warm up to RT and stirred for additional 3 h. The reaction was terminated by addition of an aqueous NH_4Cl solution. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE : EA = 50 : 1 to 10 : 1) providing the aldehyde **200** (4.3 mg, 4.4 µmol, 80%) as a colorless oil.

The analytical data are in accordance with the reported in the literature.[135]

^{1}H NMR (400 MHz, C_6D_6, C_6D_5H = 7.16 ppm) δ (ppm): 9.45 (s, 1H, H-1), 7.46 (s, 1H, Ph), 6.64 (s, 1H, Ph), 6.40 (s, 1H, Ph), 6.06 (s, 1H, NH), 5.64 (dd, *J* = 15.5, 7.4 Hz, 1H, H-6), 5.37 (dd, *J* = 15.7, 9.0 Hz, 1H, H-7), 3.97 – 3.89 (m, 1H, H-5), 3.72 – 3.70 (m, 1H, H-9), 3.72 –

3.70 (m, 1H, H-4), 3.41 – 3.31 (m, 1H, H-10), 3.31 (s, 3H, 10-OMe), 2.64 (dd, *J* = 13.2, 6.3 Hz, 1H, H-13a), 2.42 – 2.38 (m, 1H, H-13b), 2.38 – 2.35 (m, 1H, H-8), 2.33 – 2.29 (m, 1H, H-2a), 2.21 – 2.16 (m, 2H, H-2b), 2.15 – 2.10 (m, 1H, H-12), 2.02 – 1.97 (m, 1H, H-11a), 1.80 – 1.75 (m, 1H, H-3a), 1.67 – 1.63 (m, 1H, H-3b), 1.43 (s, 9H, *t*-Bu), 1.23 (d, *J* = 6.6 Hz, 3H, 8-Me), 1.19 – 1.17 (m, 1H, H-11b), 1.10 – 1.03 (m, 36H, $SiCH_2C\mathit{H}_3$), 0.97 (d, *J* = 6.5 Hz, 3H, 12-Me), 0.78 – 0.69 (m, 24H, $SiC\mathit{H}_2CH_3$); **^{13}C NMR** (100 MHz, C_6D_6, C_6D_6 = 128.06 ppm) δ (ppm): 206.1 (d, C-1), 156.7 (s, C-Ar), 152.5 (s, NHCOO), 143.2 (s, C-Ar), 139.9 (s, C-Ar), 135.2 (d, C-7), 130.3 (d, C-6), 115.9 (d, C-Ar), 110.0 (d, C-Ar), 107.7 (d, C-Ar), 81.4 (d, C-10), 79.5 (s, *t*-Bu), 78.4 (d, C-5), 76.5 (d, C-4), 76.1 (d, C-9), 57.3 (q, 10-OMe), 45.4 (t, C-13), 41.7 (d, C-8), 40.7 (t, C-2), 35.7 (t, C-11), 31.6 (d, C-12), 28.4 (q, *t*-Bu), 26.1 (t, C-3), 19.6 (q, 12-Me), 18.6 (q, 8-Me), 7.5 (q, $SiCH_2\mathit{C}H_3$), 7.4 (q, $SiCH_2\mathit{C}H_3$), 7.3 (q, $SiCH_2\mathit{C}H_3$), 7.0 (q, $SiCH_2\mathit{C}H_3$), 5.7 (t, $Si\mathit{C}H_2CH_3$), 5.6 (t, $Si\mathit{C}H_2CH_3$); **HRMS-ESI** (*m*/*z*): the compound is not detectable by mass spectrometry.

S-(2-Acetamidoethyl)-2-(triphenyl-λ^5-phosphanylidene)propanethioate (202)



213 **214** **202**

The reagent was prepared according to the Hahn's procedure[155]:

2-Bromopropanoic acid (**213**) (1.5 g, 9.8 mmol, 1.1 eq.) was dissolved in CH_2Cl_2 (25 mL) under nitrogen atmosphere, cooled down to 0 °C and treated with *N*-acetylcysteamine (0.95 mL, 8.9 mmol, 1.0 eq.) and DMAP (0.109 g, 0.891 mmol, 0.1 eq.). EDC hydrochloride (1.88 g, 9.8 mmol, 1.1 eq.) was added to the mixture portionwise. The reaction mixture was stirred at 0 °C for 1 h, warmed up to RT and stirred for another 16 h. The reaction was terminated by addition of an aqueous $NaHCO_3$ solution. The phases were separated and the organic phase was washed with H_2O and NaCl solution and dried over $MgSO_4$. The solvent was concentrated under reduced pressure to give the product **214** (2.36 g, 9.3 mmol, 94%) as a pale yellow oil which was used in the next step without further purification.

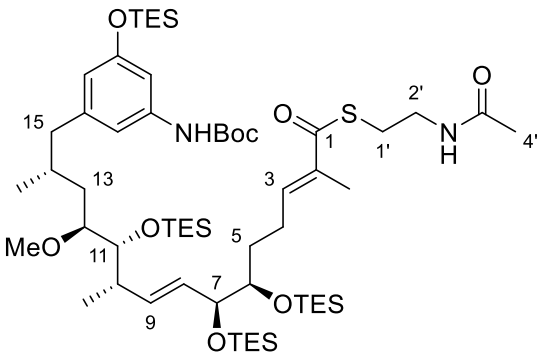
^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 6.09 (bs, 1H, NH), 4.50 (q, *J* = 6.9 Hz, 1H, C*H*Br), 3.46 – 3.39 (m, 2H, $C\mathit{H}_2NH$), 3.13 – 2.98 (m, 2H, $C\mathit{H}_2S$), 1.95 (s, 3H, CH_3CO), 1.81 (d, *J* = 6.9 Hz, 3H, $C\mathit{H}_3CHBr$); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 196.8 (s, C(=O)S), 170.5 (s, C(=O)N), 47.9 (d, CHBr), 39.2 (t, CH_2NH), 29.4

(t, CH_2S), 23.3 (q, *C*H_3CO), 22.0 (q, *C*H_3CHBr); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_7H_{12}NO_2SBrNa]^+$: 275.9670, found: 275.9669.

S-(2-Acetamidoethyl) 2-bromopropanethioate (**214**) (2.3 g, 9.0 mmol, 1.1 eq.) was suspended in H_2O (12 mL) and treated with triphenylphosphine (2.15 g, 8.2 mmol, 1.0 eq.). The reaction mixture was stirred at 70 °C for 11 h. After that time the solution was cooled down to RT, treated with NaOH (0.66 g, 16.5 mmol, 2.0 eq.) dissolved in H_2O (20 mL) and the mixture was stirred for 5 min. The aqueous phase was extracted three times with CH_2Cl_2, the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was recrystallized from toluene and washed with cold solvent to give colorless crystalline product **202** (1.9 g, 4.5 mmol, 50%).

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm) δ (ppm): 7.60 – 7.47 (m, 15H, Ar), 3.34 – 3.31 (m, 2H, C*H*$_2$NH), 3.02 – 2.99 (m, 2H, C*H*$_2$S), 1.68 (d, *J* = 6.9 Hz, C*H*$_3$CP), 1.54 (s, 3H, C*H*$_3$CO); **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 180.5 (s, C(=O)S), 170.5 (s, C(=O)N), 133.7 , 133.6 (d, 6C, C-Ar), 132.3 (d, 3C, C-Ar), 129.1 (d, 6C, C-Ar), 125.4 (s, 3C, C-Ar), 53.7, 52.4 (s, *C*=PPh_3), 43.4 (t, CH_2NH), 26.9 (t, CH_2S), 23.2 (q, *C*H_3CO), 13.3 (q, *C*H_3CP); **HRMS-ESI** (*m*/*z*): $[M+H]^+$ calcd for $[C_{25}H_{27}NO_2SP]^+$: 436.1500, found: 436.1495.

Silylated SNAc ester 201



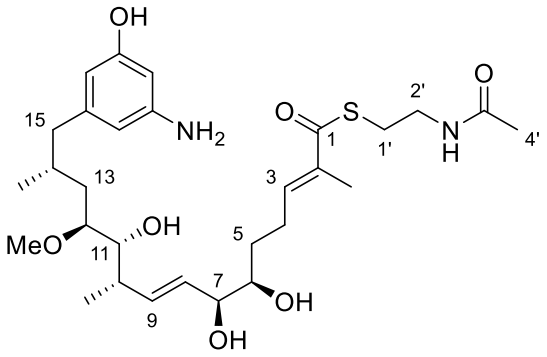
201

$C_{58}H_{110}N_2O_9SSi_4$
Mol. Wt.: 1123.92 g/mol

The aldehyde **200** (4.0 mg, 4.14 µmol, 1.0 eq.) was dissolved in $CHCl_3$ (0.5 mL), treated with phosphonium ylide **202** (9.0 mg, 20.7 µmol, 5.0 eq.) and the reaction mixture was stirred at 40 °C for 2 days. After that time the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (PE : EA = 7 : 1 to 1 : 1) providing the SNAc ester **201** (4.0 mg, 3.56 µmol, 86%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$, $CDCl_3$ = 7.26 ppm) δ (ppm): 6.79 (s, 1H, Ph), 6.74 – 6.72 (m, 1H, H-3), 6.70 (s, 1H, Ph), 6.45 (s, 1H, Ph), 5.91 (s, 1H, NH), 5.49 (dd, *J* = 15.5, 7.1 Hz, 1H, H-9), 5.38 (dd, *J* = 15.5, 8.7 Hz, 1H, H-8), 3.92 (dd, *J* = 7.0, 2.4 Hz, 1H, H-7), 3.68 – 3.61 (m, 1H, H-6), 3.56 (dd, *J* = 9.0, 1.4 Hz, 1H, H-11), 3.42 (dd, *J* = 12.3, 6.1 Hz, 2H, H-2'), 3.25 (s, 3H, 12-OMe), 3.19 (d, *J* = 10.6 Hz, 1H, H-12), 3.05 (t, *J* = 6.4 Hz, 2H, H-1'), 2.53 (dd, *J* = 13.3, 5.9 Hz, 1H, H-15a), 2.39 – 2.31 (m, 1H, H-4a), 2.35 (dd, *J* = 13.3, 5.9 Hz, 1H, H-15b), 2.22 – 2.18 (m, 1H, H-10), 2.16 – 2.09 (m, 1H, H-4b), 1.95 (s, 3H, H-4'), 1.92 – 1.86 (m, 1H, H-14), 1.85 (s, 3H, 2-Me), 1.70 – 1.67 (m, 1H, H-13a), 1.66 – 1.63 (m, 1H, H-5a), 1.54 – 1.52 (m, 1H, H-5b), 1.57 (s, 9H, *t*-Bu), 1.09– 1.06 (m, 1H, H-13b), 1.05 (d, *J* = 6.7 Hz, 3H, 10-Me), 1.00 – 0.92 (m, 36H, $SiCH_2C\mathit{H}_3$), 0.79 (d, *J* = 6.5 Hz, 3H, 14-Me), 0.75 – 0.56 (m, 24H, $SiC\mathit{H}_2CH_3$); **^{13}C NMR** (125 MHz, $CDCl_3$, $CDCl_3$ = 77.16 ppm) δ (ppm): 194.1 (s, C-1), 170.4 (s, C-3'), 156.0 (s, C-Ar), 152.7 (s, NHCOO), 143.5 (d, C-3), 142.1 (s, C-Ar), 139.2 (s, C-Ar), 136.0 (d, C-9), 134.9 (s, C-2), 130.3 (d, C-8), 115.7 (d, C-Ar), 112.5 (d, C-Ar), 108.7 (d, C-Ar), 81.1 (d, C-7), 77.9 (d, C-12), 77.4 (s, *t*-Bu), 76.5 (d, C-6), 75.9 (d, C-11), 57.4 (q, 12-OMe), 45.0 (t, C-15), 40.9 (t, C-2'), 40.0 (d, C-10), 35.7 (t, C-13), 32.3 (t, C-5), 31.2 (d, C-14), 28.5 (q, *t*-Bu), 28.5 (t, C-1'), 25.6 (t, C-4), 23.4 (q, C-4'), 19.2 (q, 14-Me), 18.0 (q, 10-Me), 12.6 (q, 2-Me), 7.2 (q, $SiCH_2\mathit{C}H_3$), 7.0 (q, $SiCH_2\mathit{C}H_3$), 6.8 (q, $SiCH_2\mathit{C}H_3$), 5.3 (t, $Si\mathit{C}H_2CH_3$), 5.2 (t, $Si\mathit{C}H_2CH_3$); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{58}H_{110}N_2O_9SSi_4Na]^+$: 1145.6907, found: 1145.6904.

SNAc ester 137



$C_{29}H_{46}N_2O_7S$
Mol. Wt.: 566.75 g/mol

137

The silylated SNAc ester **201** (3.5 mg, 3.1 µmol, 1.0 eq.) was dissolved in CH_2Cl_2 (3.0 mL) under nitrogen atmosphere, cooled down to 0 °C and treated with TFA (300 µL). After 3 h the reaction was terminated by addition of an aqueous $NaHCO_3$ solution, the phases were separated and the aqueous phase was extracted three times with EtOAc. The combined

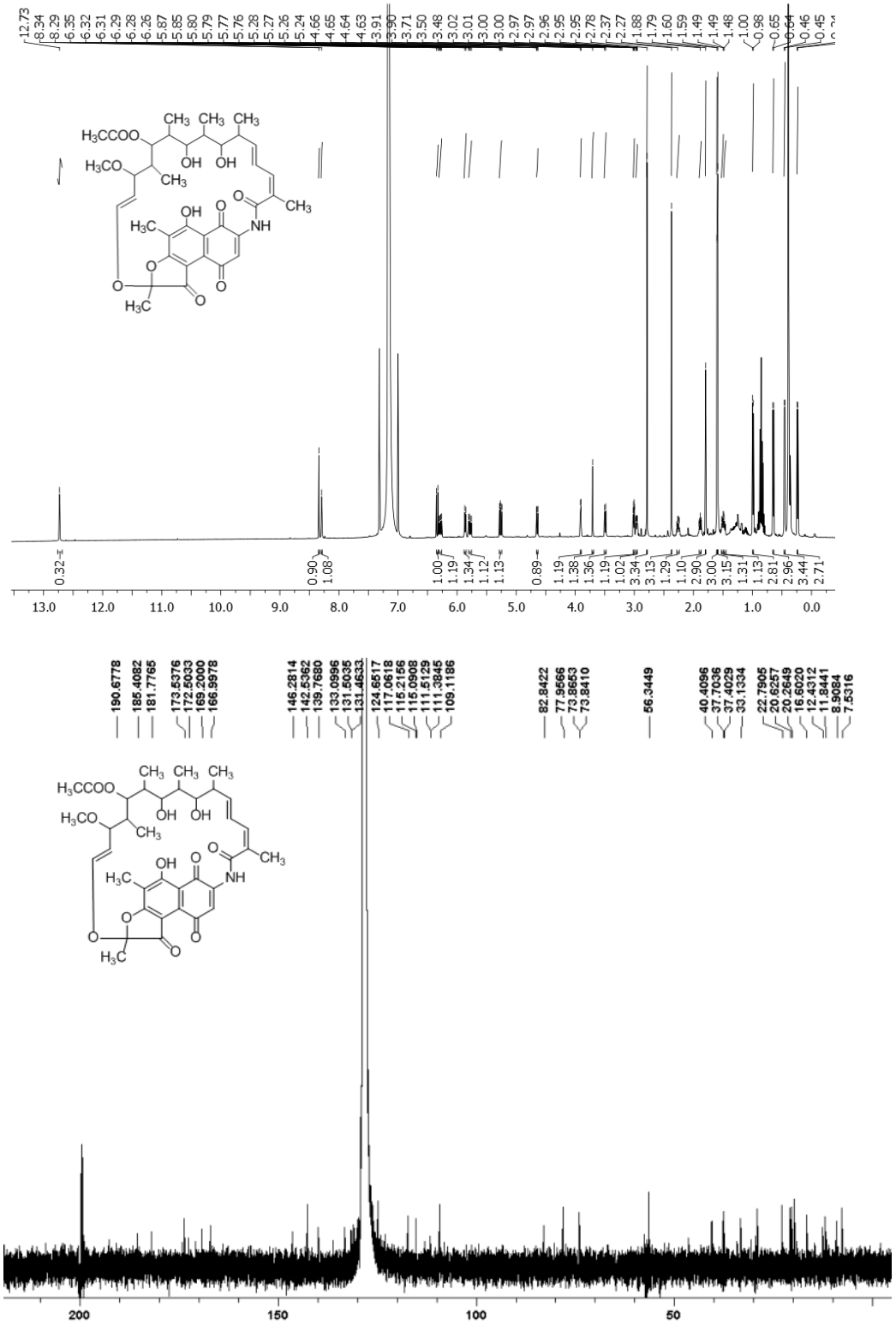
organic phases were washed with an aqueous NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by semi-preparative HPLC (solvent A: water + 0.1% (v/v) formic acid, solvent B: MeOH + 0.1% (v/v) formic acid; flow rate = 2.5 mL/min; gradient (*t* [min]/solvent B [%]): 0/30; 5/30; 85/60; 90/85, 100/100; t_R = 20 min) providing the SNAc ester **137** (1.3 mg, 2.2 µmol, 73%) as a colorless oil.

^{1}H NMR (500 MHz, CD_3OD, CHD_2OD = 3.31 ppm) δ (ppm): 6.81 – 6.78 (m, 1H, H-3), 6.72 (s, 1H, Ph), 6.65 (m, 1H, Ph), 6.62 (s, 1H, Ph), 5.60 – 5.59 (m, 1H, H-9), 5.60 – 5.59 (m, 1H, H-8), 3.94 – 3.92 (m, 1H, H-7), 3.60 (d, *J* = 3.0 Hz, 1H, H-11), 3.58 (d, *J* = 3.0 Hz, 1H, H-6), 3.51 – 3.48 (m, 2H, H-2'), 3.35 (s, 3H, 12-OMe), 3.20 – 3.16 (s, 1H, H-12), 3.04 – 3.01 (t, *J* = 6.9 Hz, 2H, H-1'), 2.57 (dd, *J* = 13.4, 7.1 Hz, 1H, H-15a), 2.46 (dd, *J* = 13.4, 7.5 Hz, 1H, H-15b), 2.36 – 2. 19 (m, 1H, H-4a), 2.36 – 2. 19 (m, 1H, H-4b), 2.36 – 2. 19 (m, 1H, H-10), 2.00 – 1.97 (m, 1H, H-14), 1.94 (s, 3H, H-4'), 1.88 (s, 3H, 2-Me), 1.71 – 1.64 (m, 1H, H-13a), 1.71 – 1.64 (m, 1H, H-5a), 1.57 – 1.52 (m, 1H, H-5b), 1.24 – 1.22 (m, 1H, H-13b), 1.12 (d, *J* = 6.7 Hz, 3H, 10-Me), 0.89 (d, *J* = 6.4 Hz, 3H, 14-Me); **^{13}C NMR** (125 MHz, CD_3OD, CD_3OD = 49.0 ppm) δ (ppm): 194.5 (s, C-1), 173.5 (s, C-3'), 156.0 (s, C-Ar), 153.7 (s, C-Ar), 146.4 (d, C-3), 142.4 (s, C-Ar), 137.4 (s, C-2), 136.4 (d, C-9), 130.9 (d, C-8), 108.4 (d, C-Ar), 107.2 (d, C-Ar), 103.0 (d, C-Ar), 81.4 (d, C-7), 76.9 (d, C-12), 75.0 (d, C-6), 74.8 (d, C-11), 57.1 (q, 12-OMe), 45.3 (t, C-15), 41.1 (t, C-2'), 40.3 (d, C-10), 35.9 (t, C-13), 32.5 (t, C-5), 32.3 (d, C-14), 29.0 (t, C-1'), 26.1 (t, C-4), 22.5 (q, C-4'), 19.7 (q, 14-Me), 18.1 (q, 10-Me), 12.5 (q, 2-Me); **HRMS-ESI** (*m*/*z*): $[M+Na]^+$ calcd for $[C_{29}H_{46}N_2O_7NaS]^+$: 589.2923, found: 589.2924.

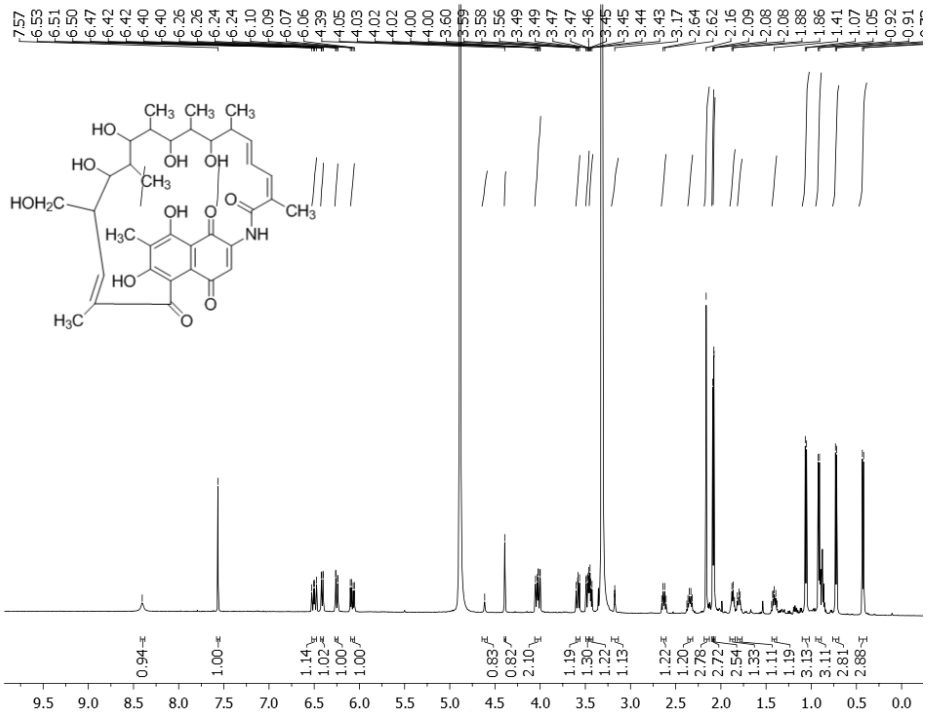
4 Appendix

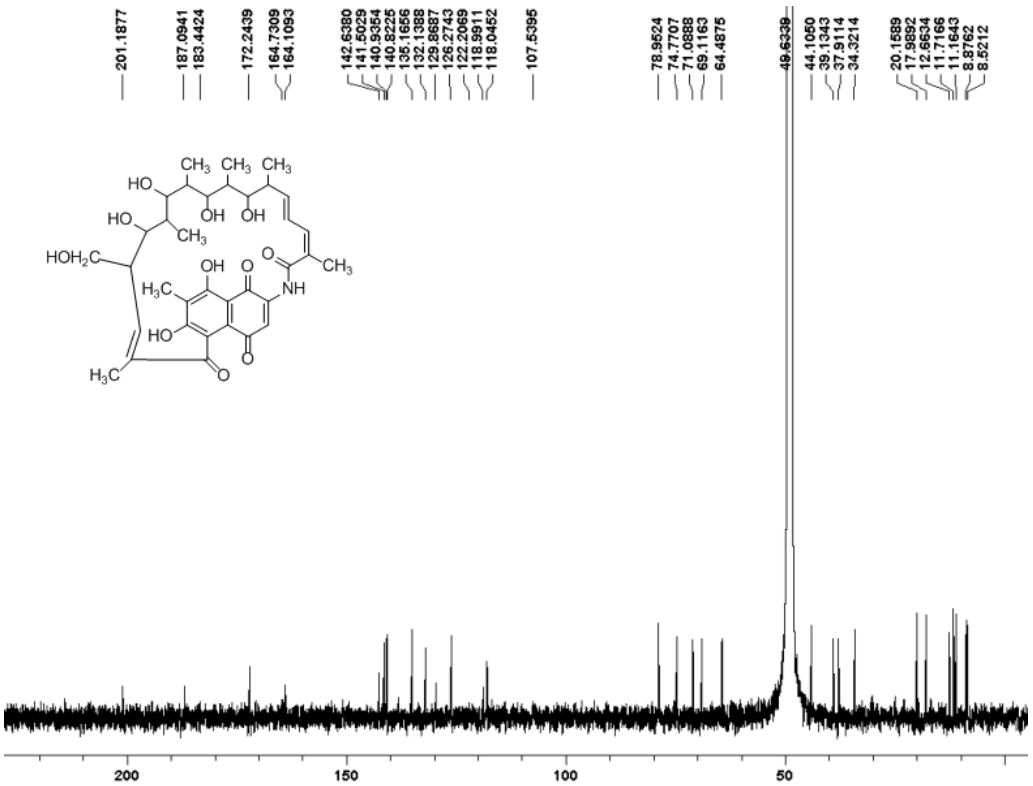
4.1 NMR Spectra

Rifamycin S (9)

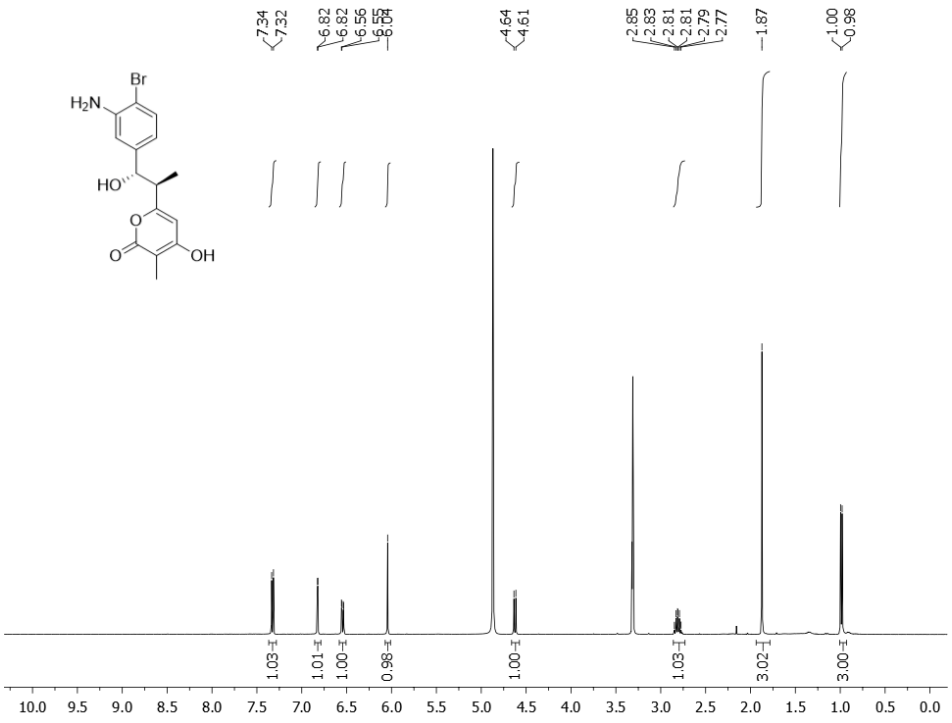


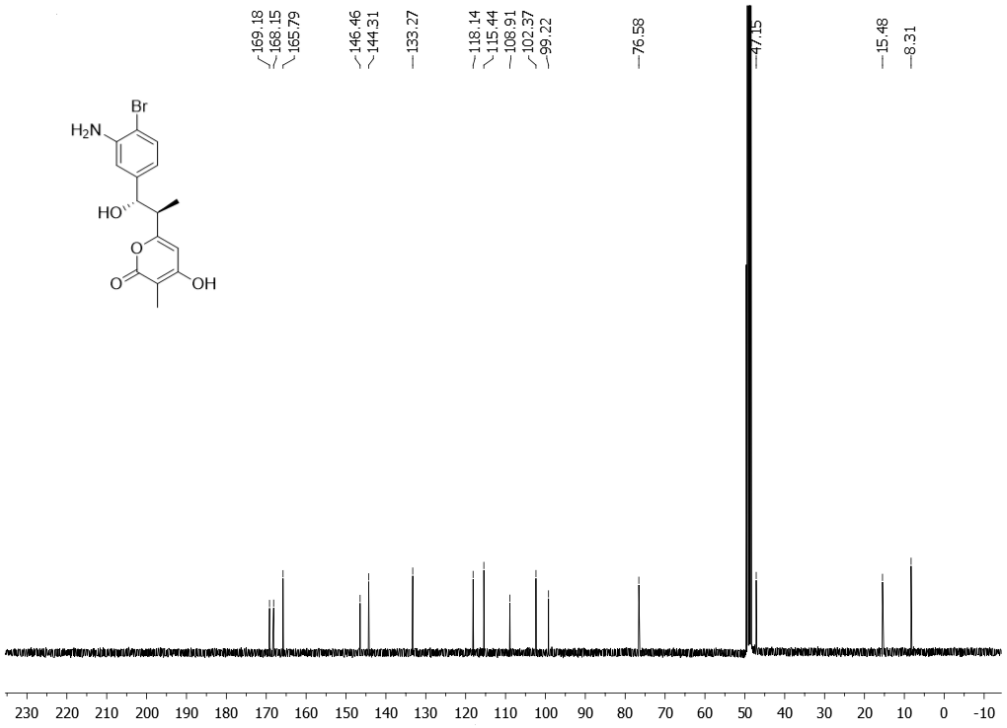
Rifamycin W (24)



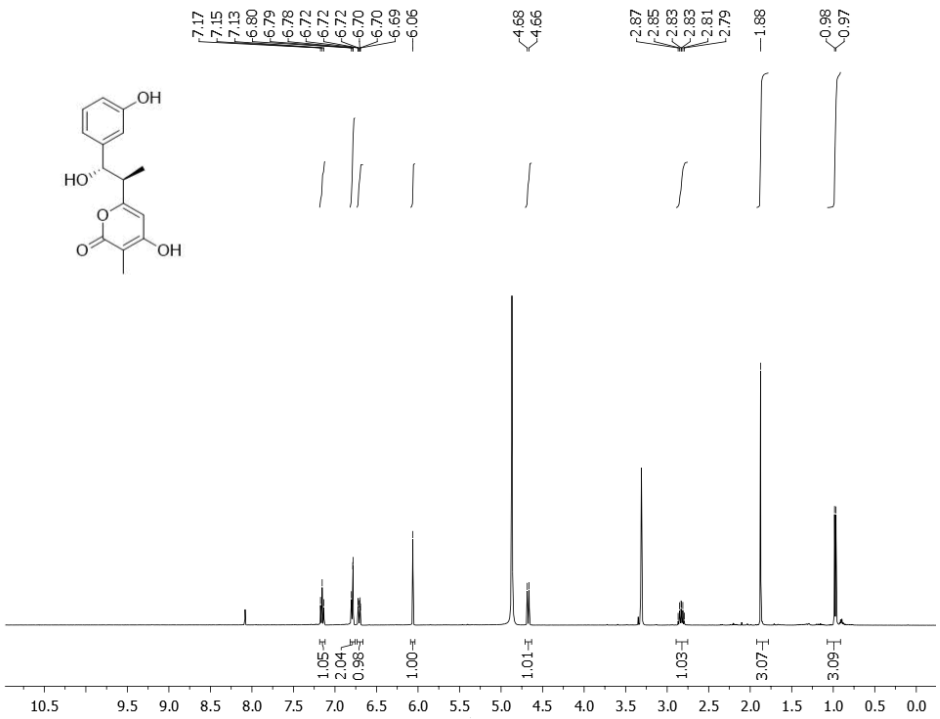


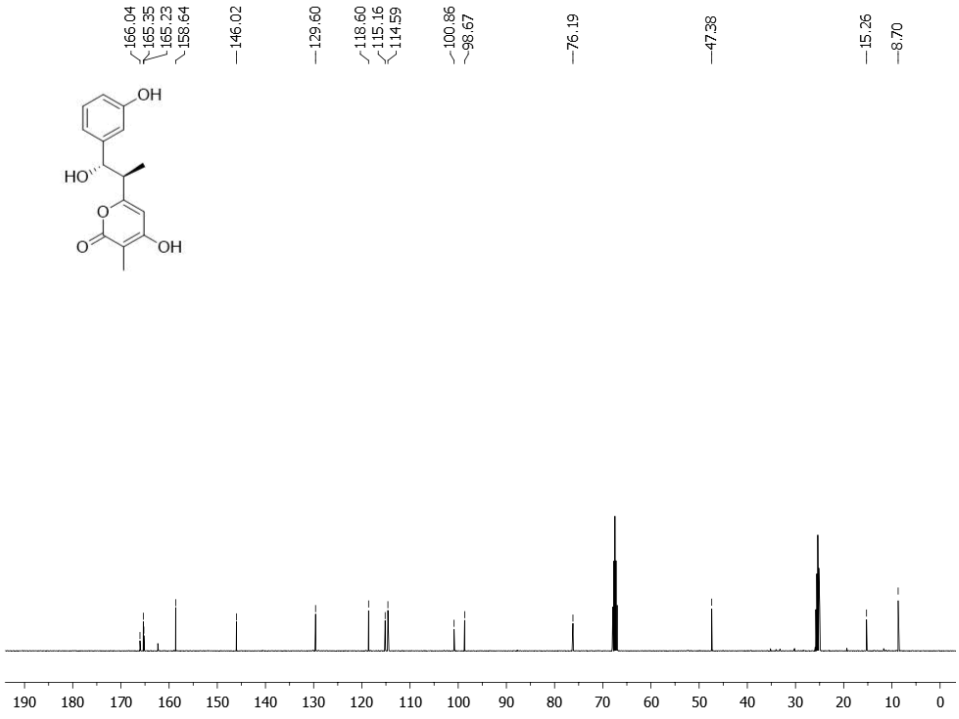
Tetraketide 42



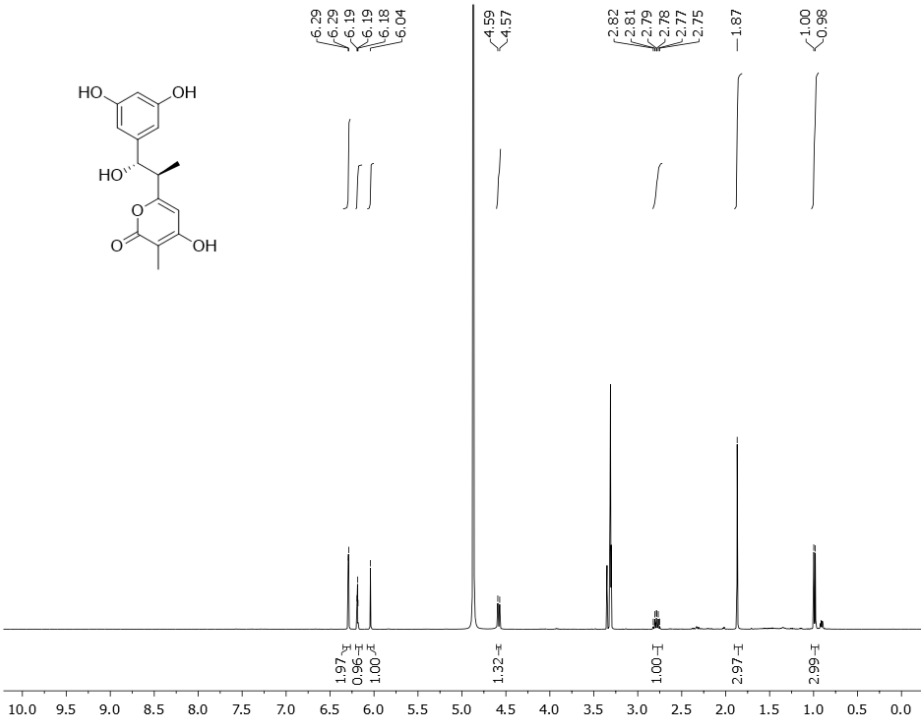


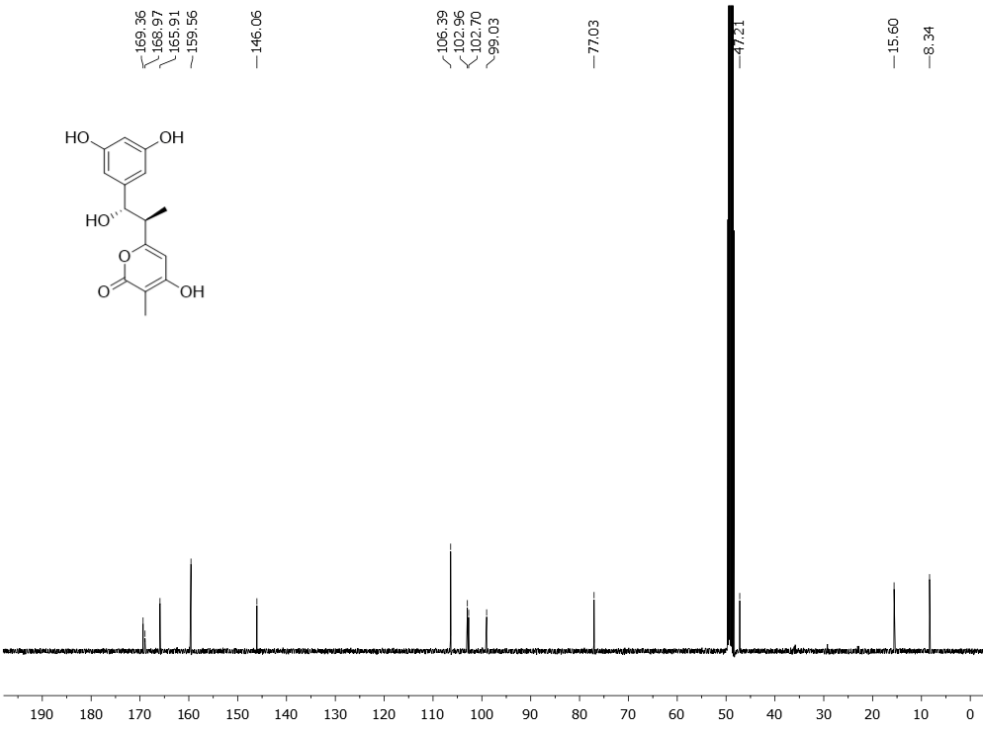
Tetraketide 67



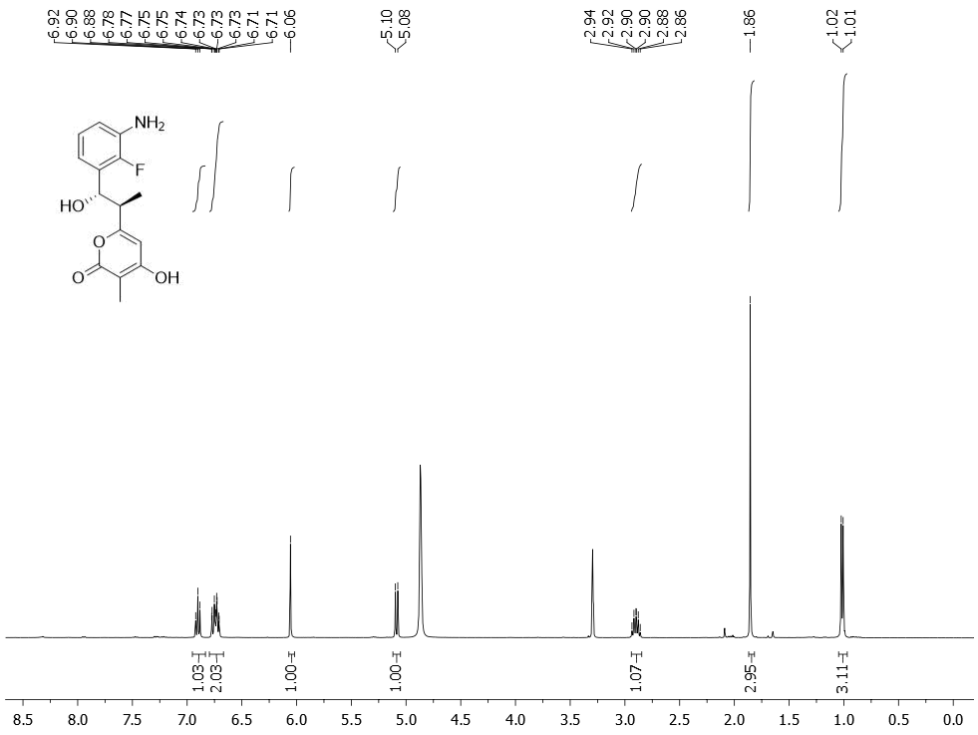


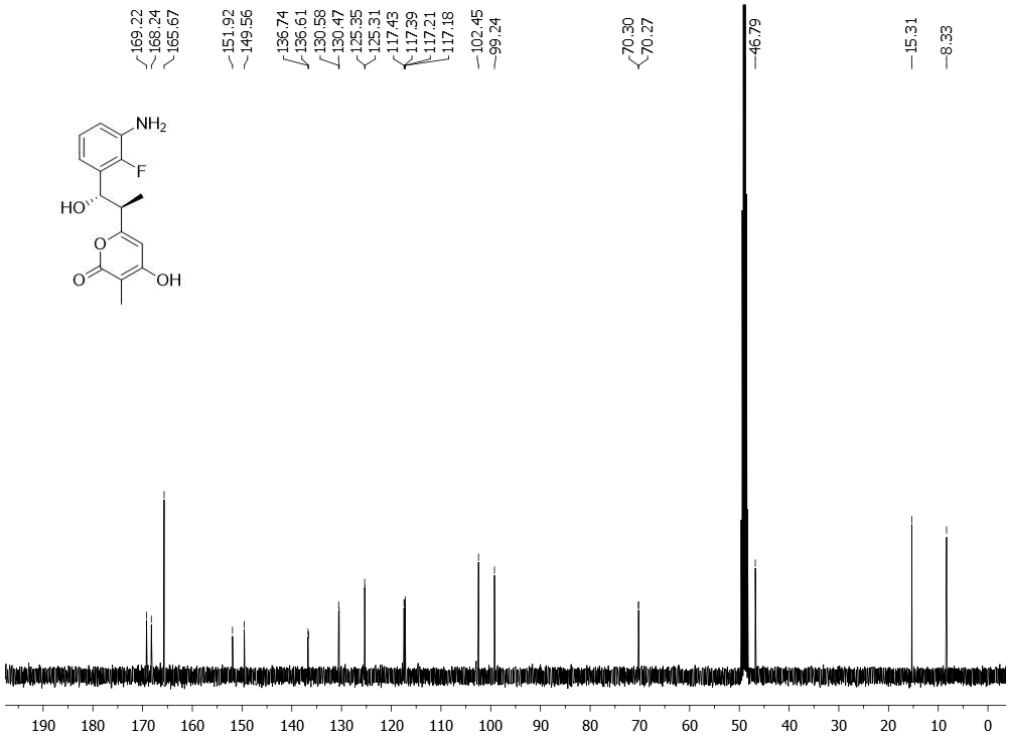
Tetraketide 68



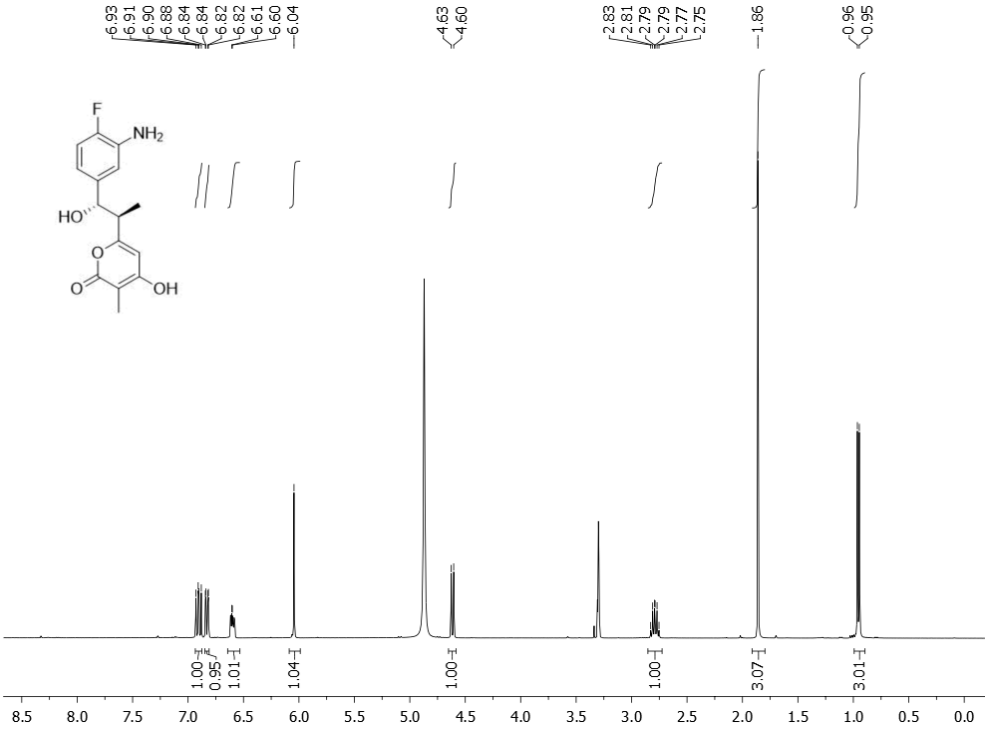


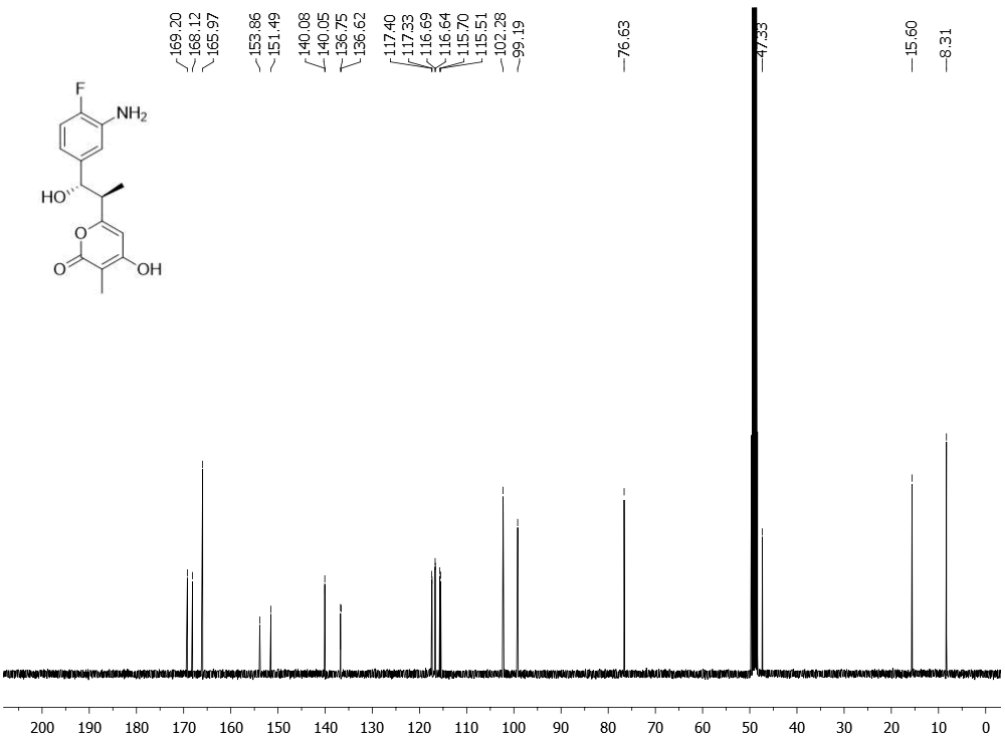
Tetraketide 87



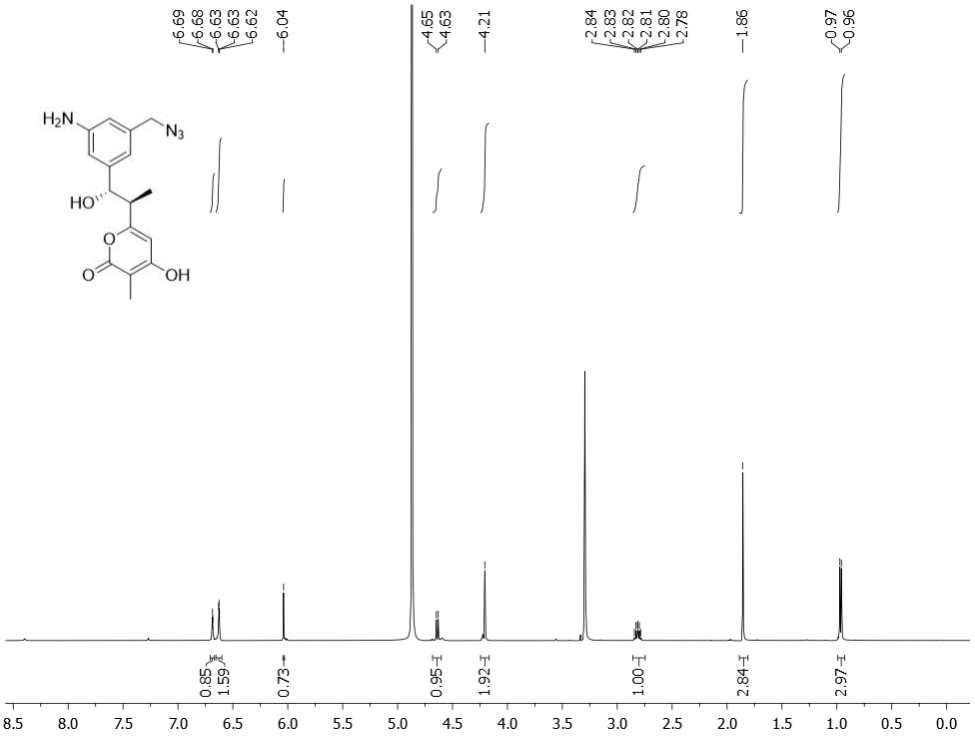


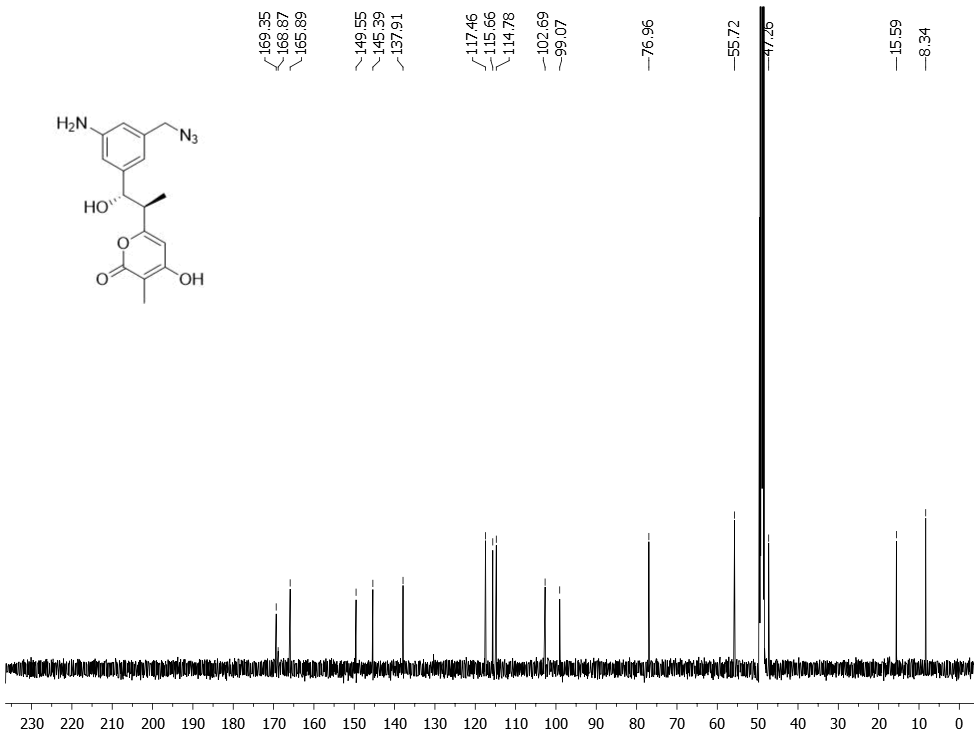
Tetraketide 88



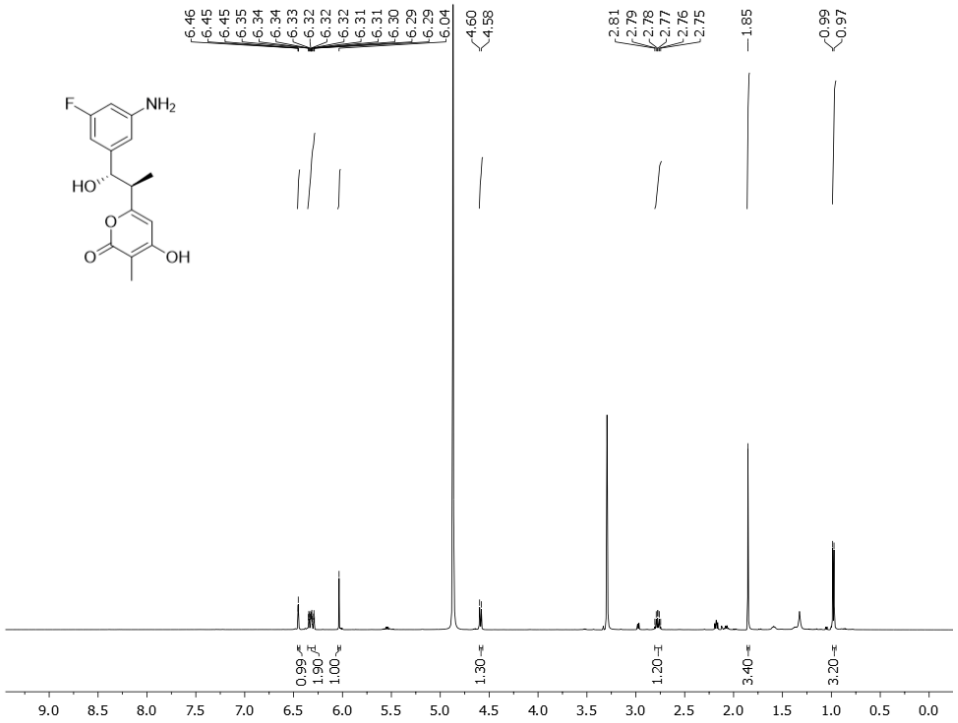


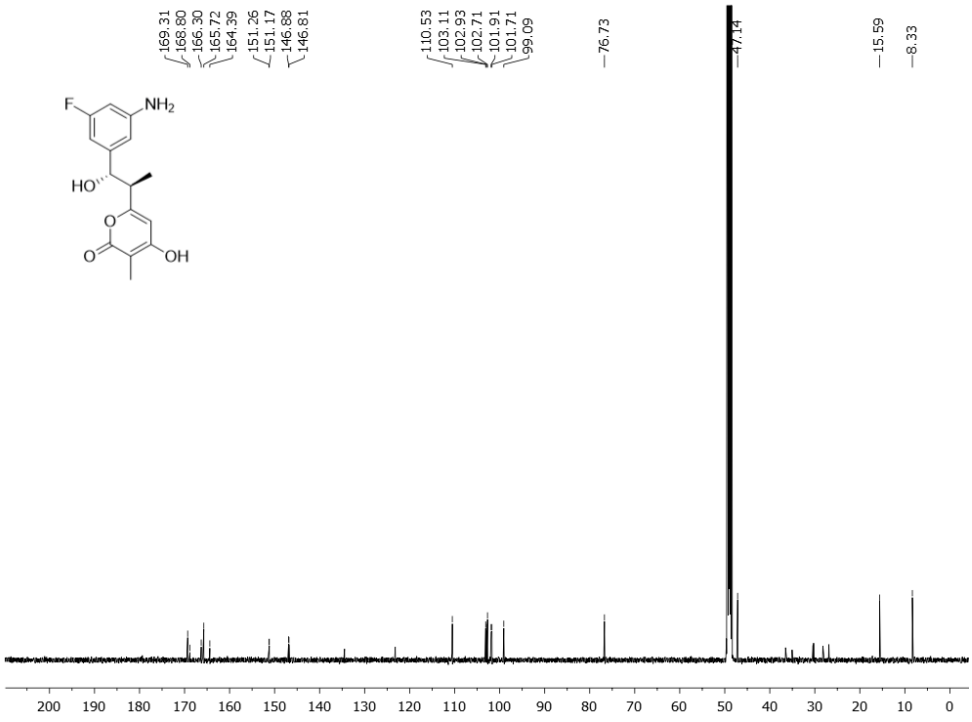
Tetraketide 89



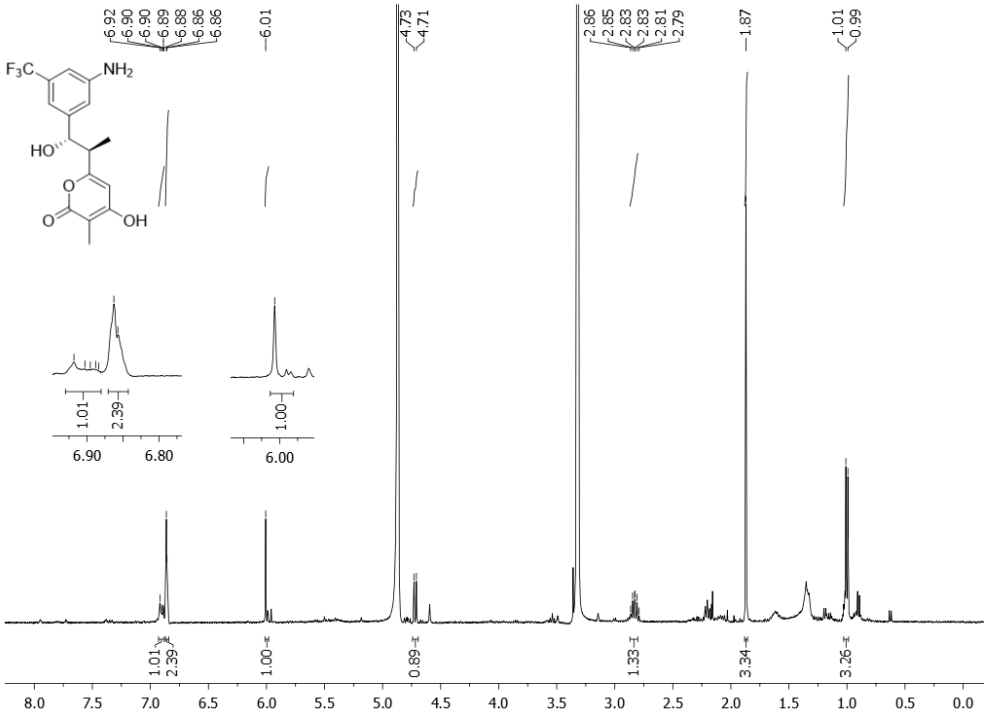


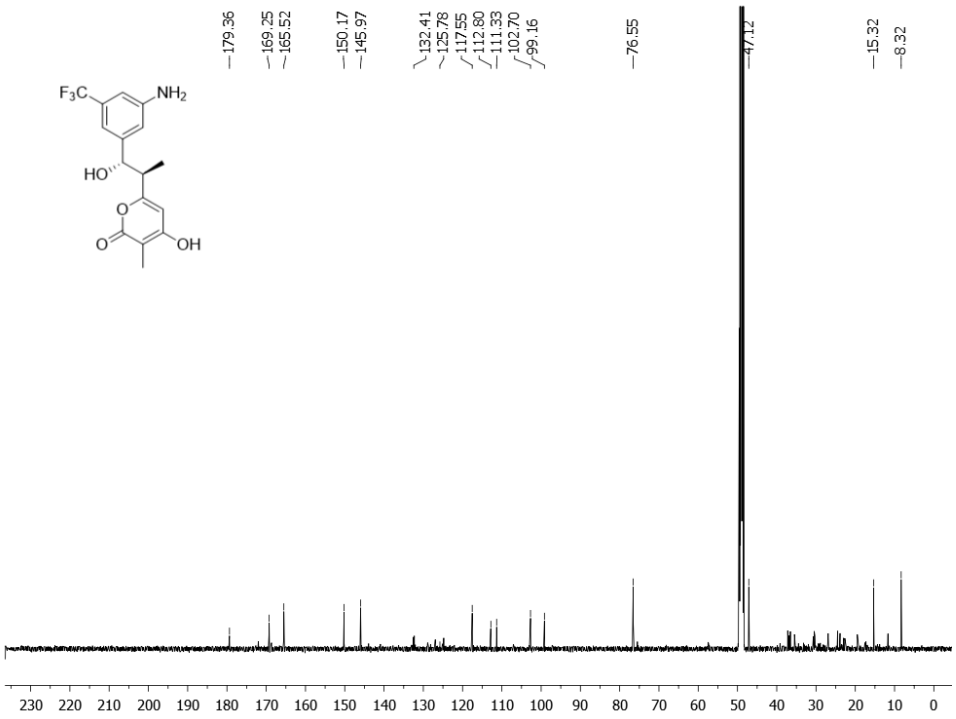
Tetraketide 90



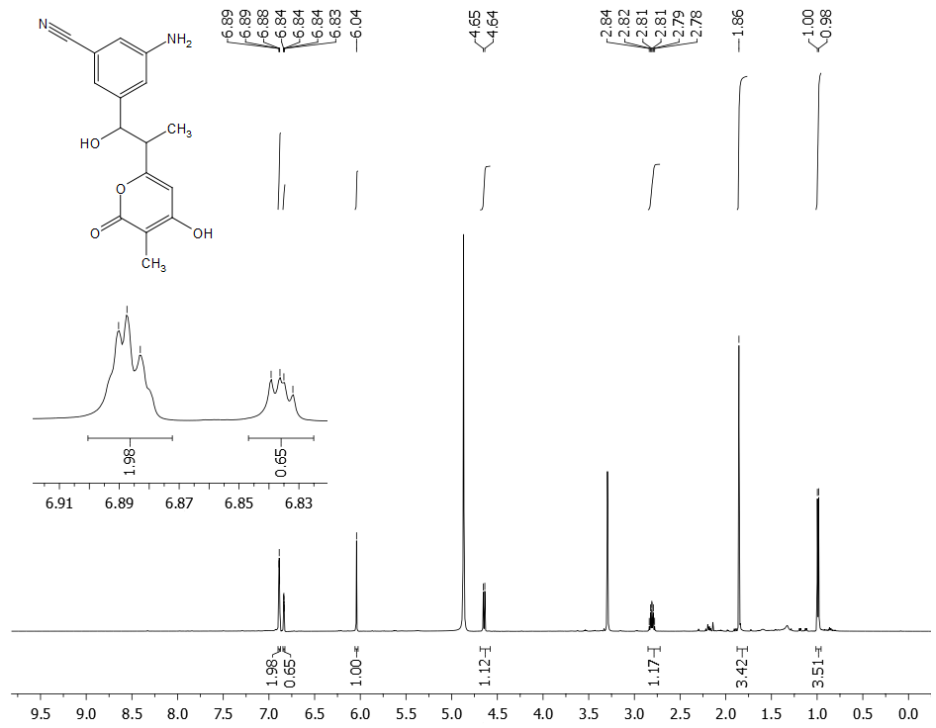


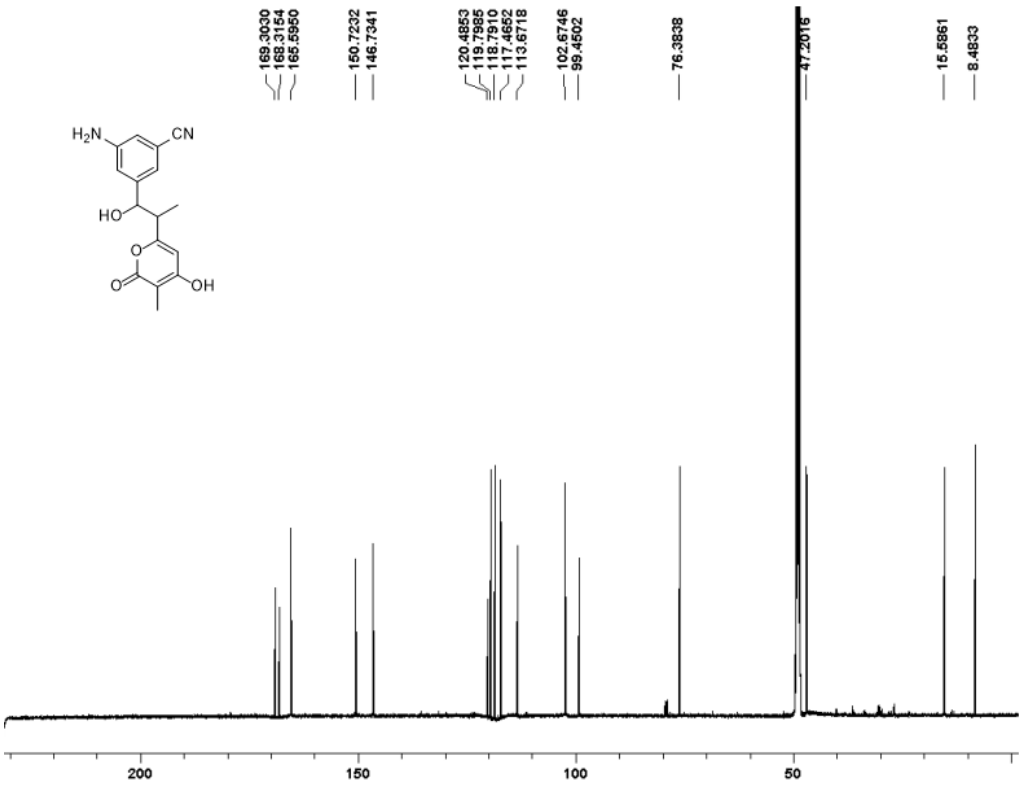
Tetraketide 91



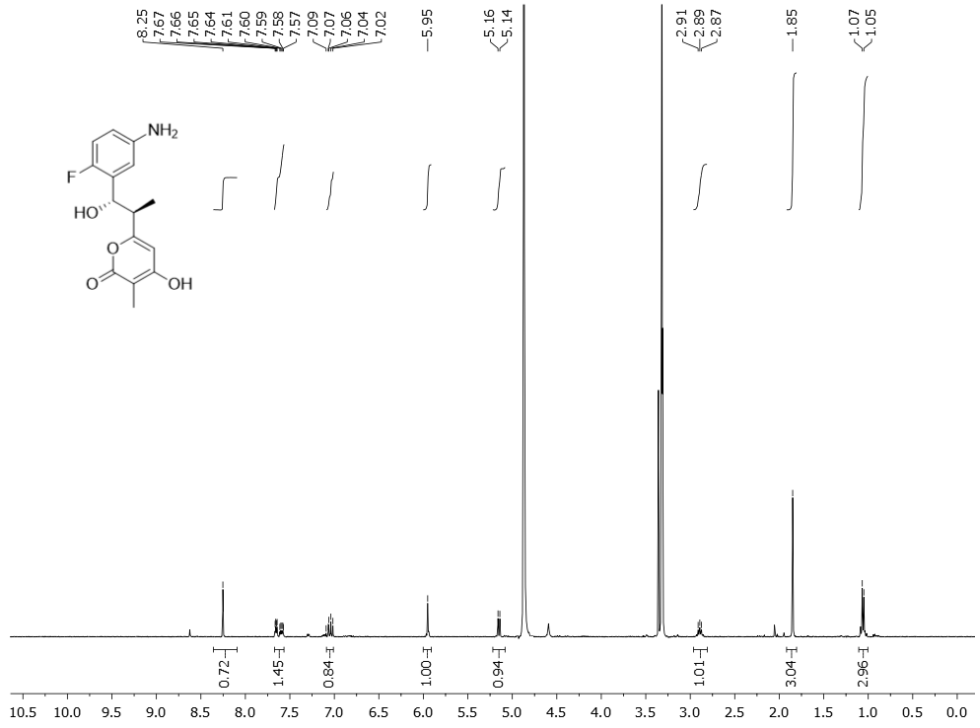


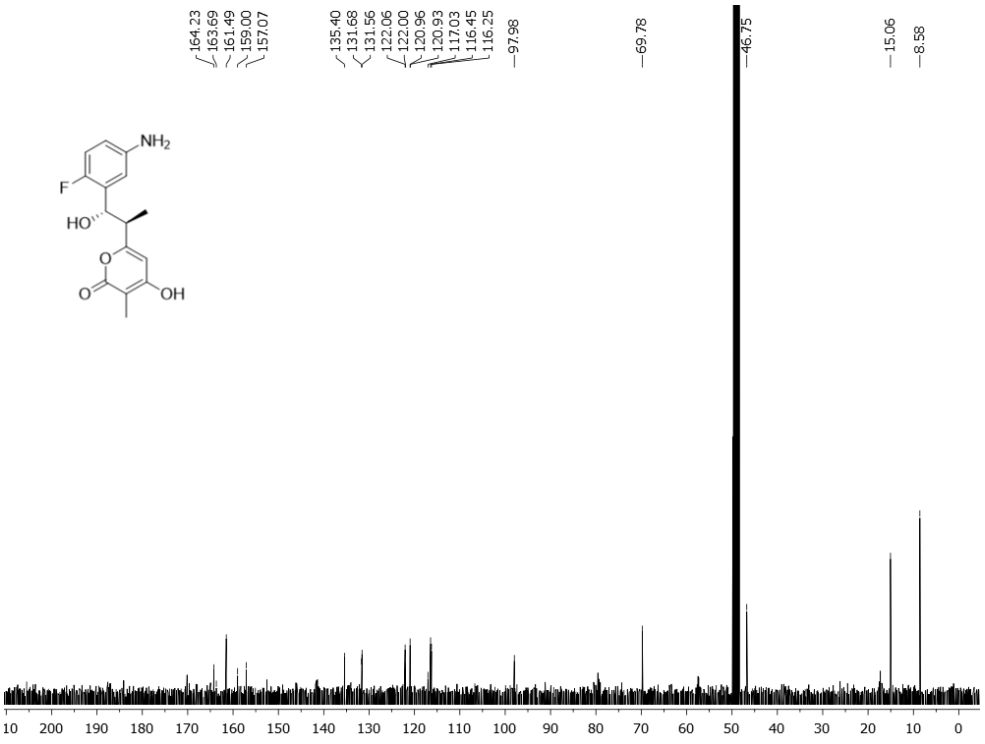
Tetraketide 92



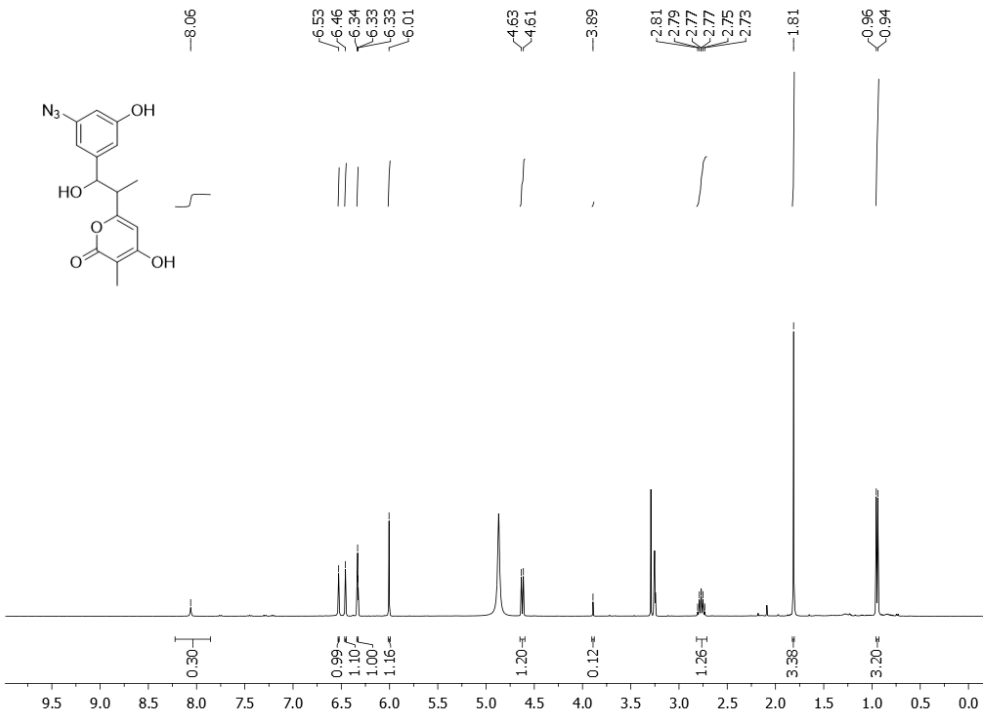


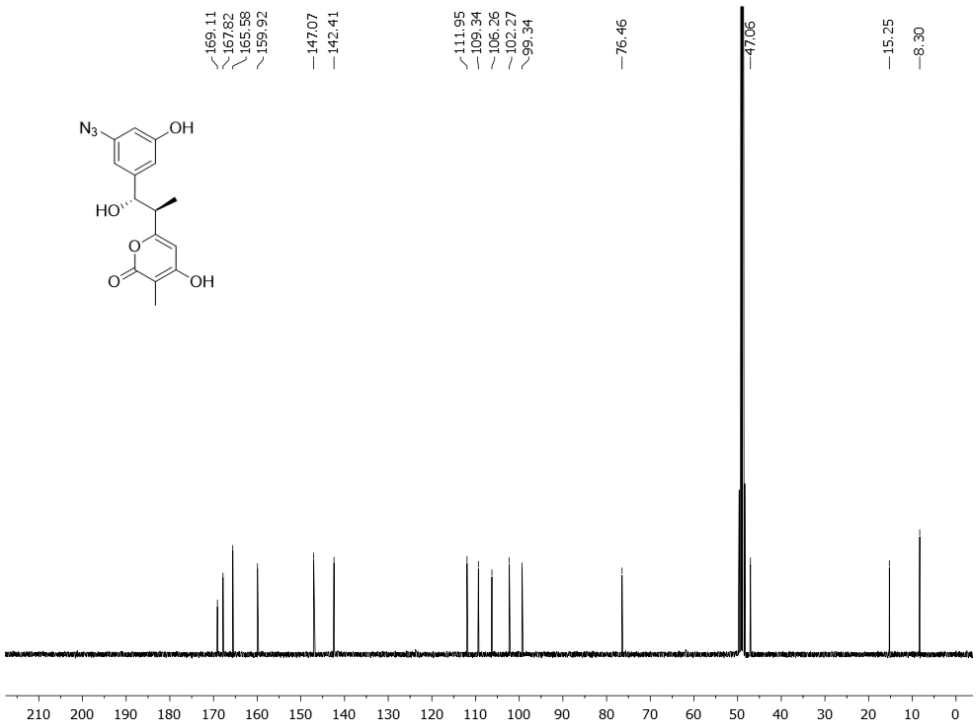
Tetraketide 93



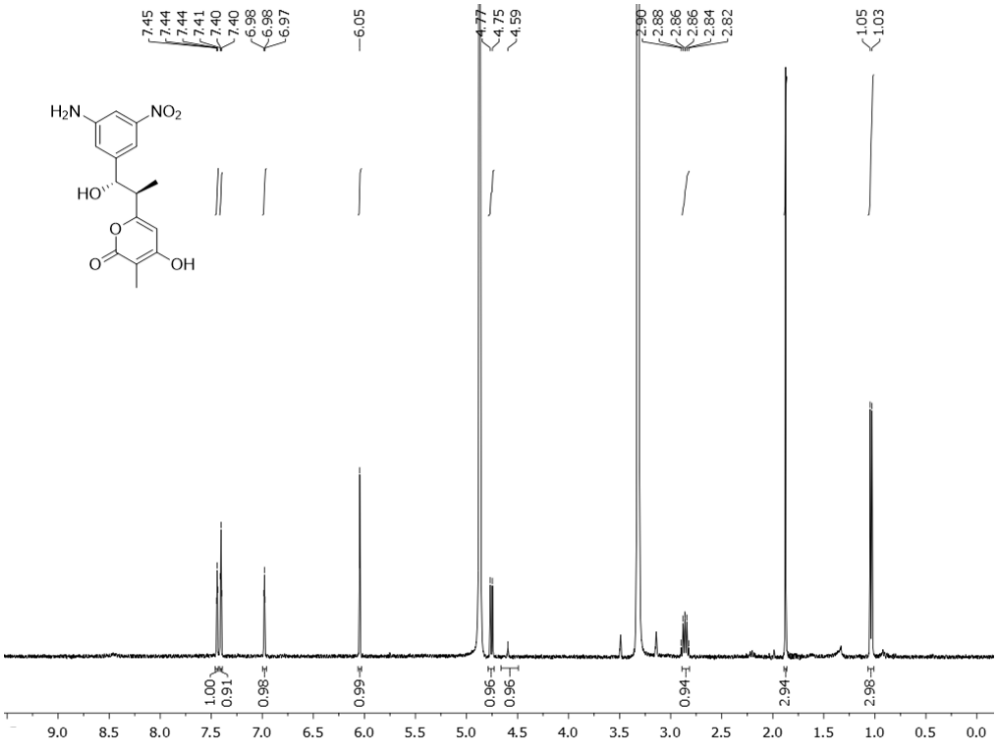


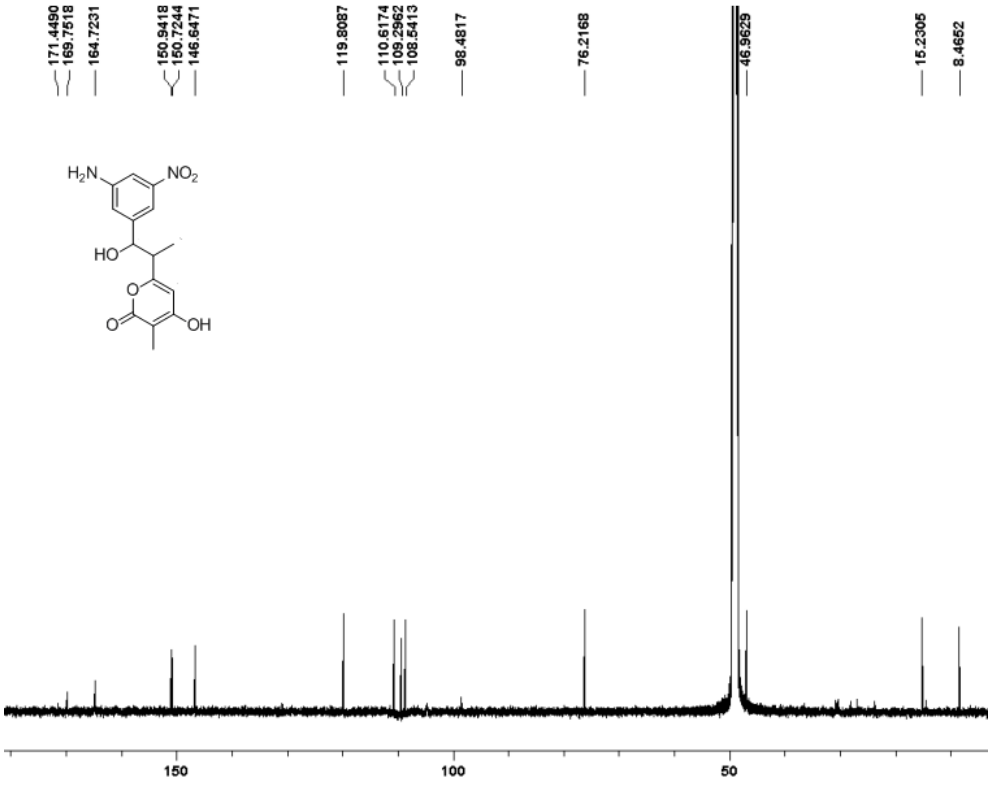
Tetraketide 96



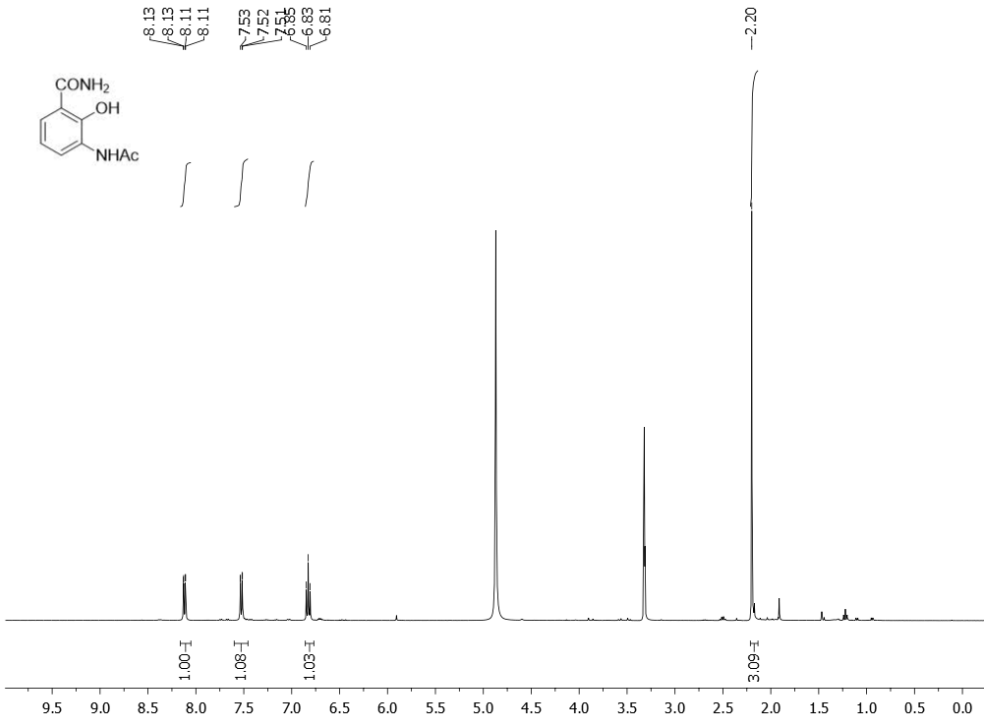


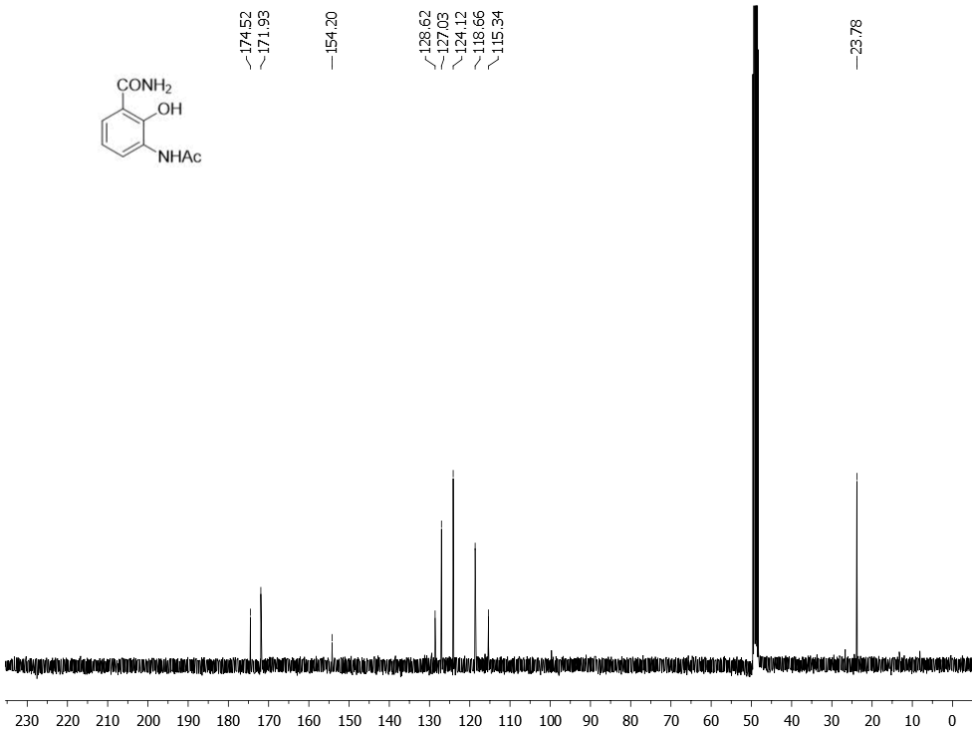
Tetraketide 102



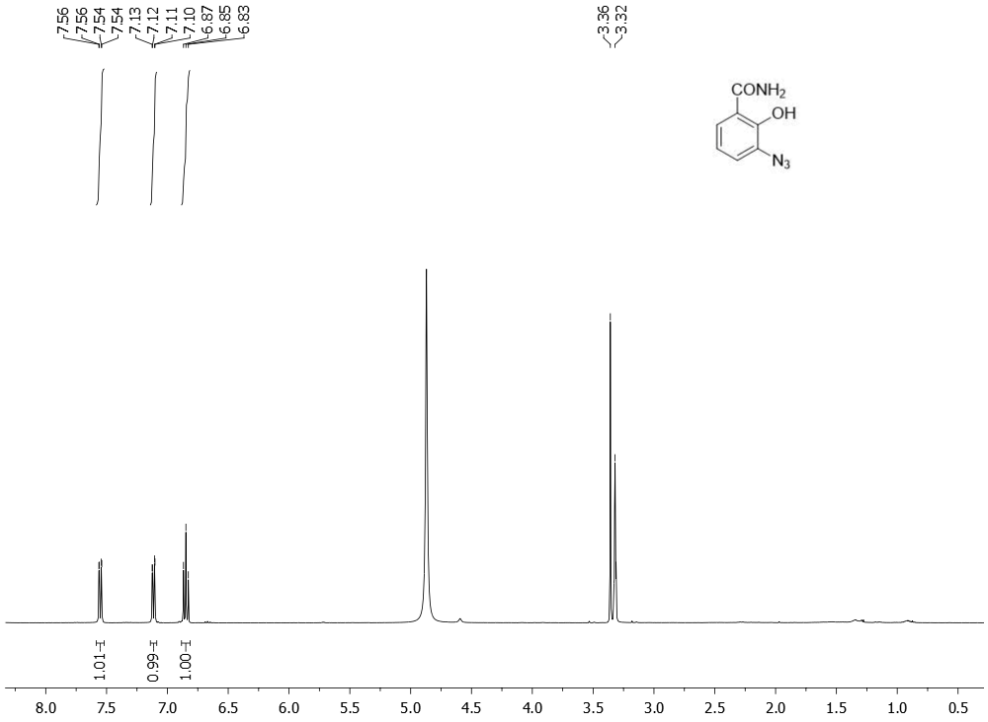


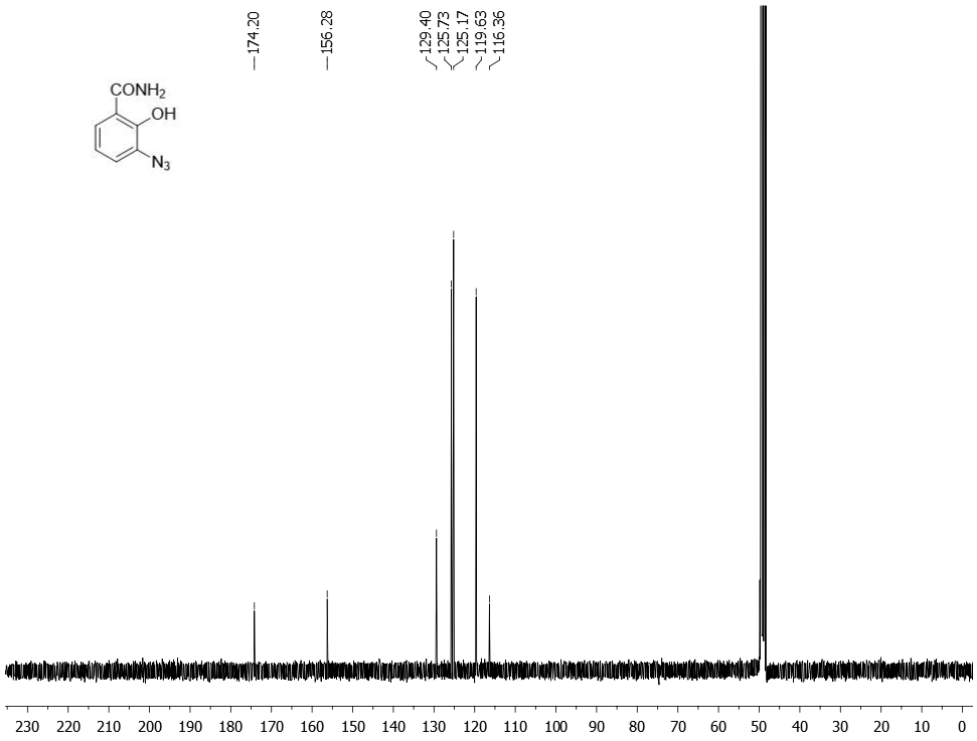
3-Acetamido-2-hydroxybenzamide (103)



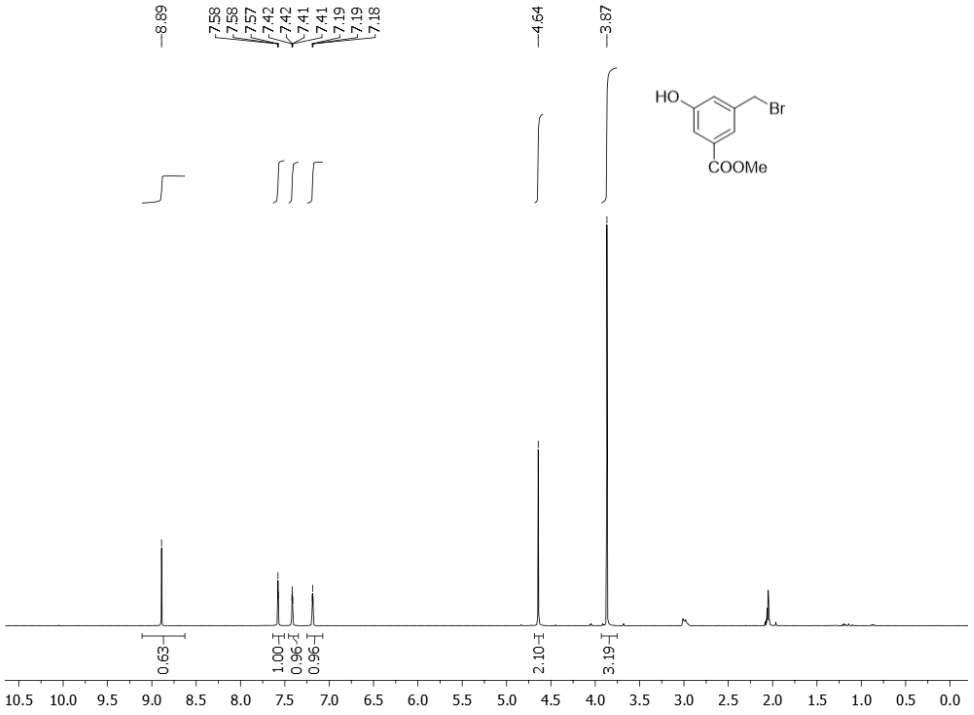


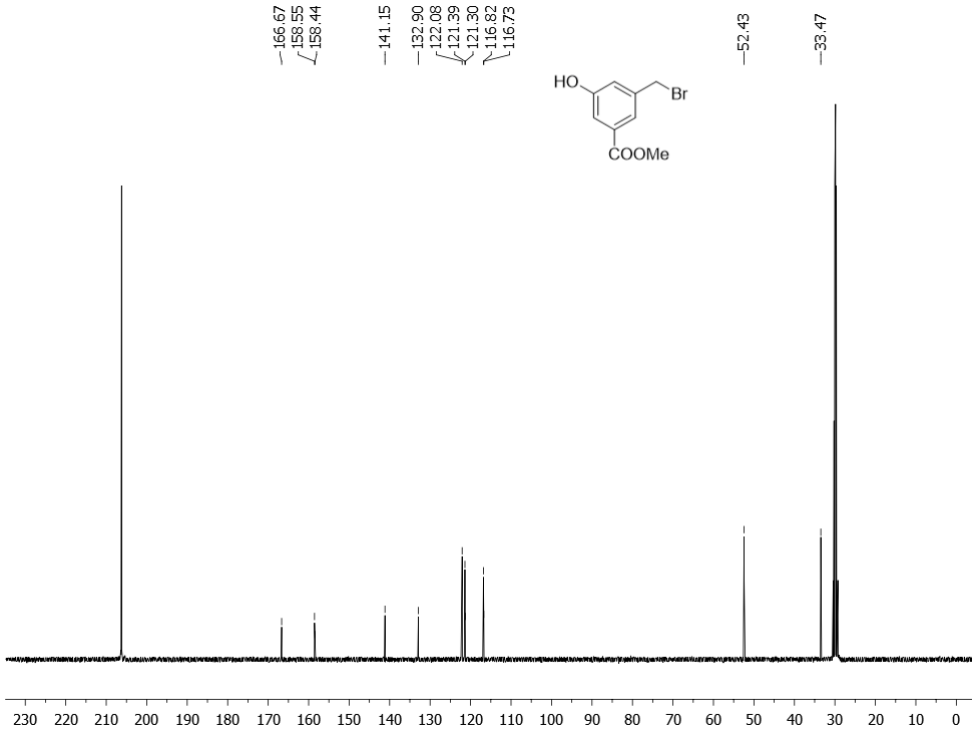
3-Azido-2-hydroxybenzamide (104)



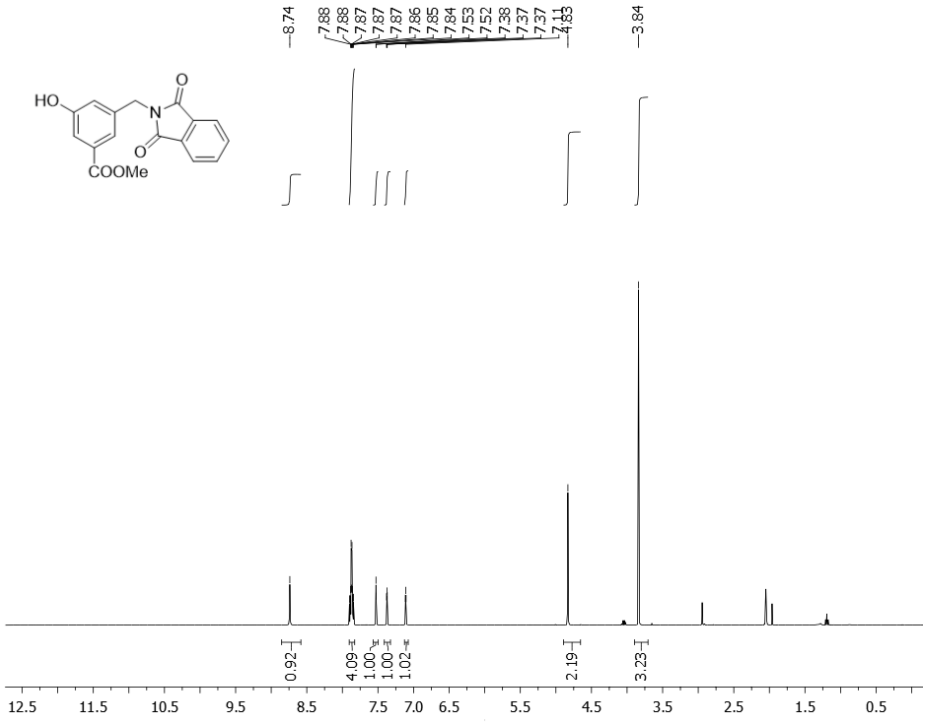


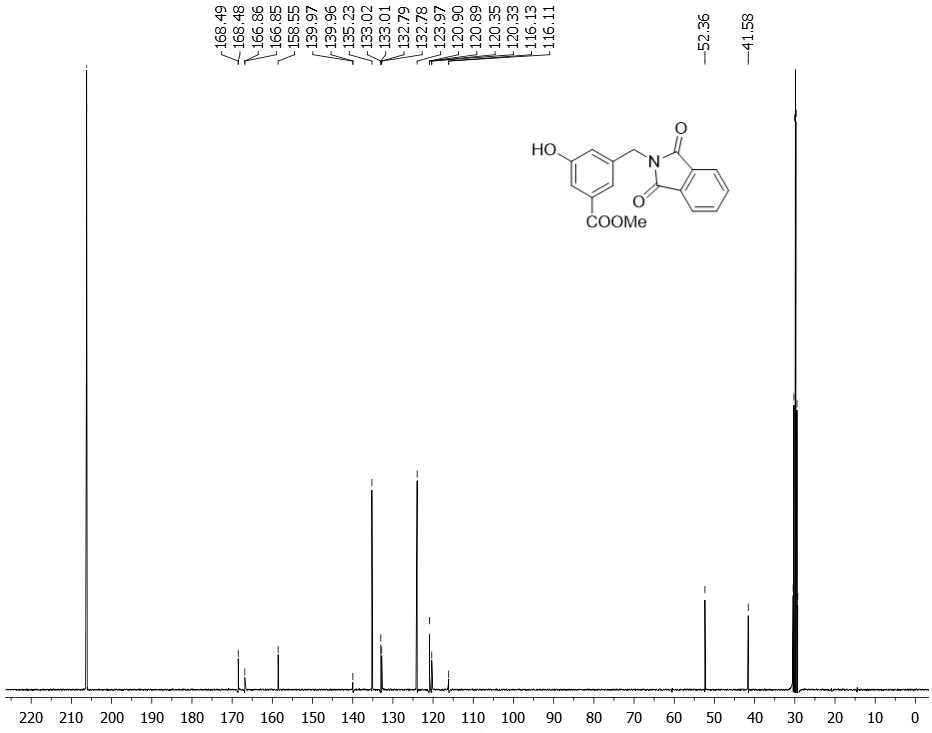
Methyl 3-(bromomethyl)-5-hydroxybenzoate (49)



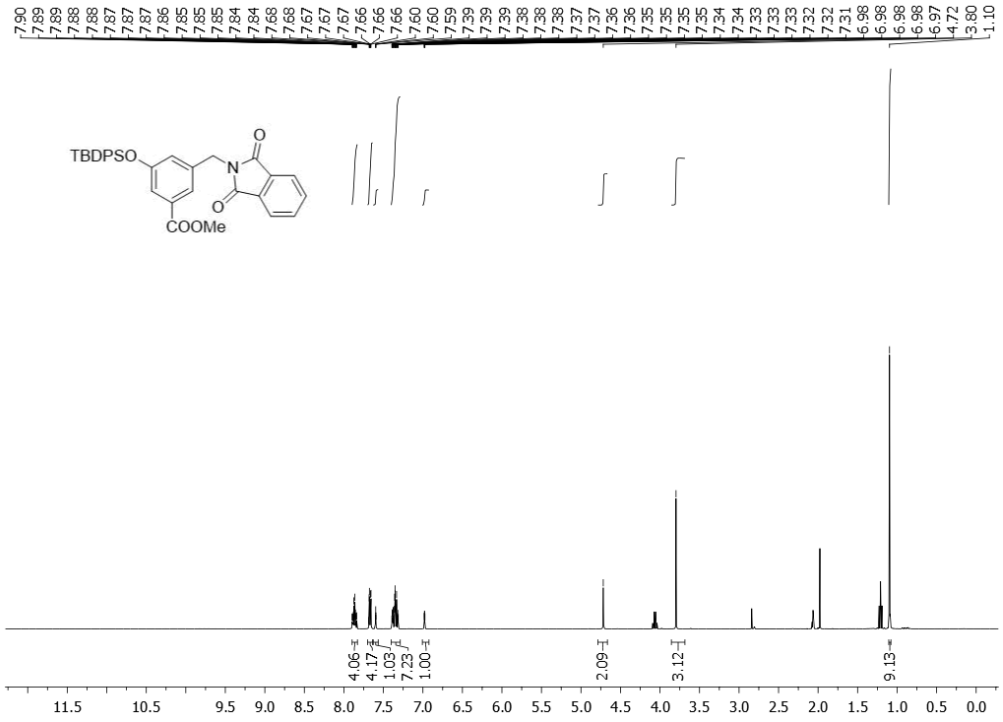


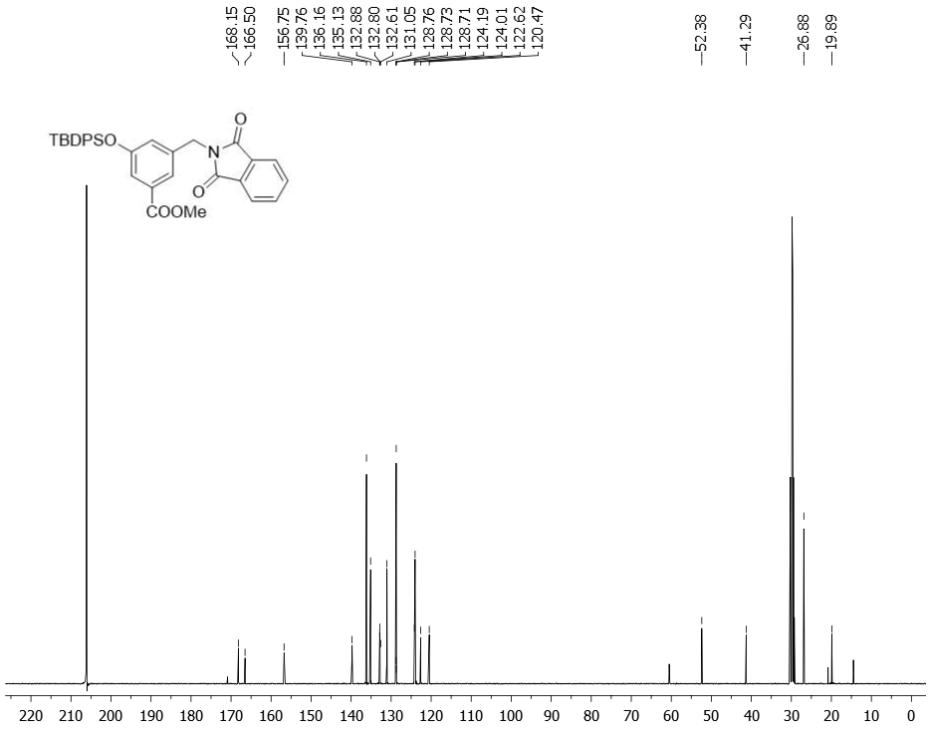
Methyl 3-[(1,3-dioxoisoindolin-2-yl)methyl]-5-hydroxybenzoate (52)



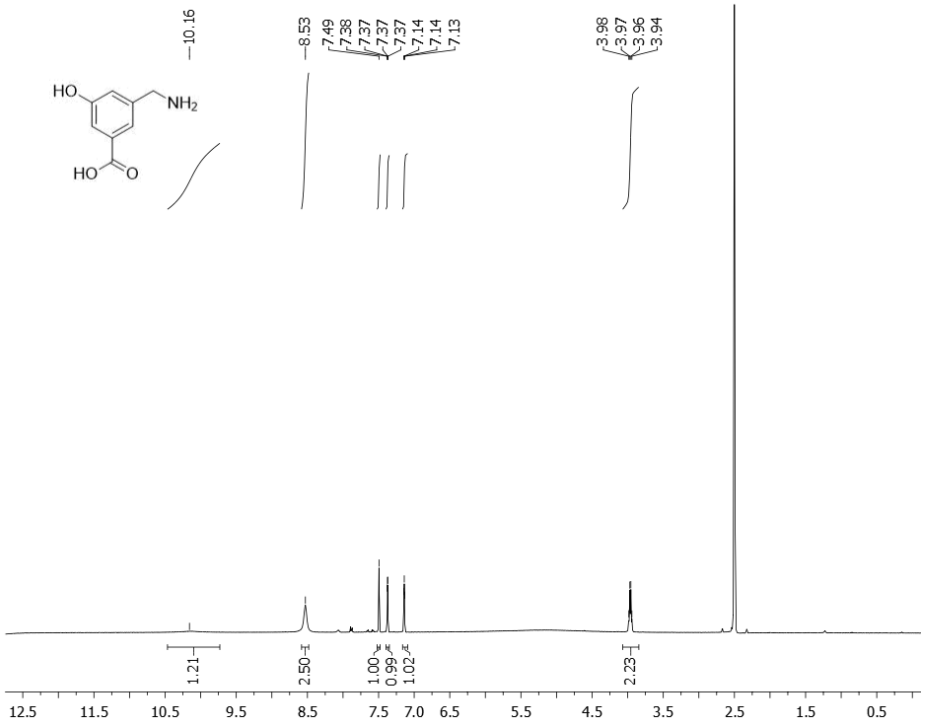


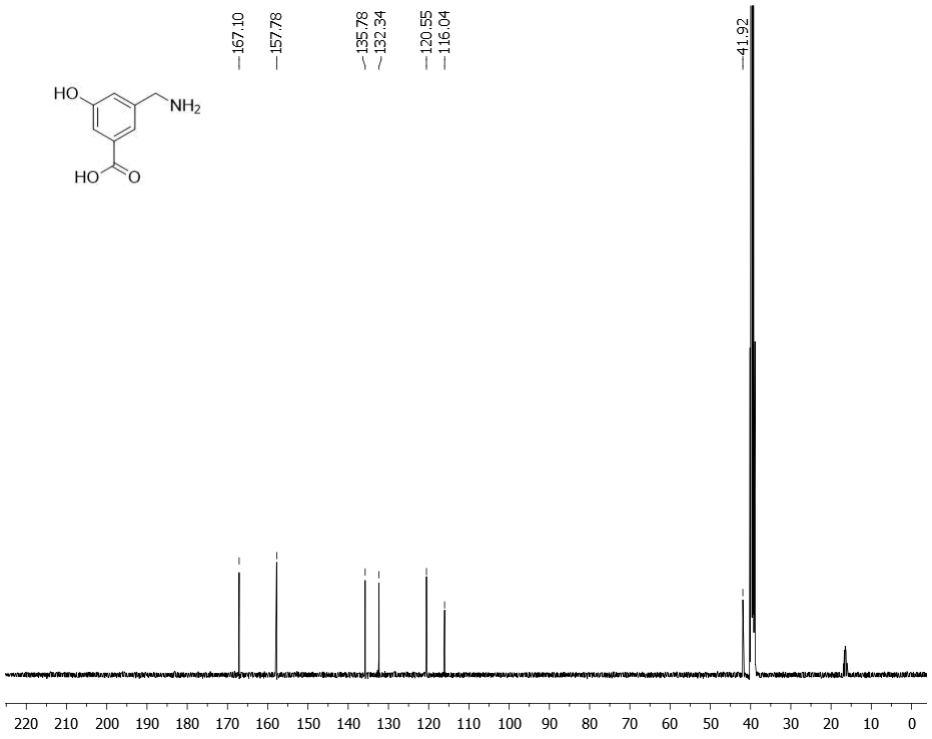
Methyl 3-[(*tert*-butyldiphenylsilyl)oxy]-5-[(1,3-dioxoisoindolin-2-yl)methyl]benzoate (51)



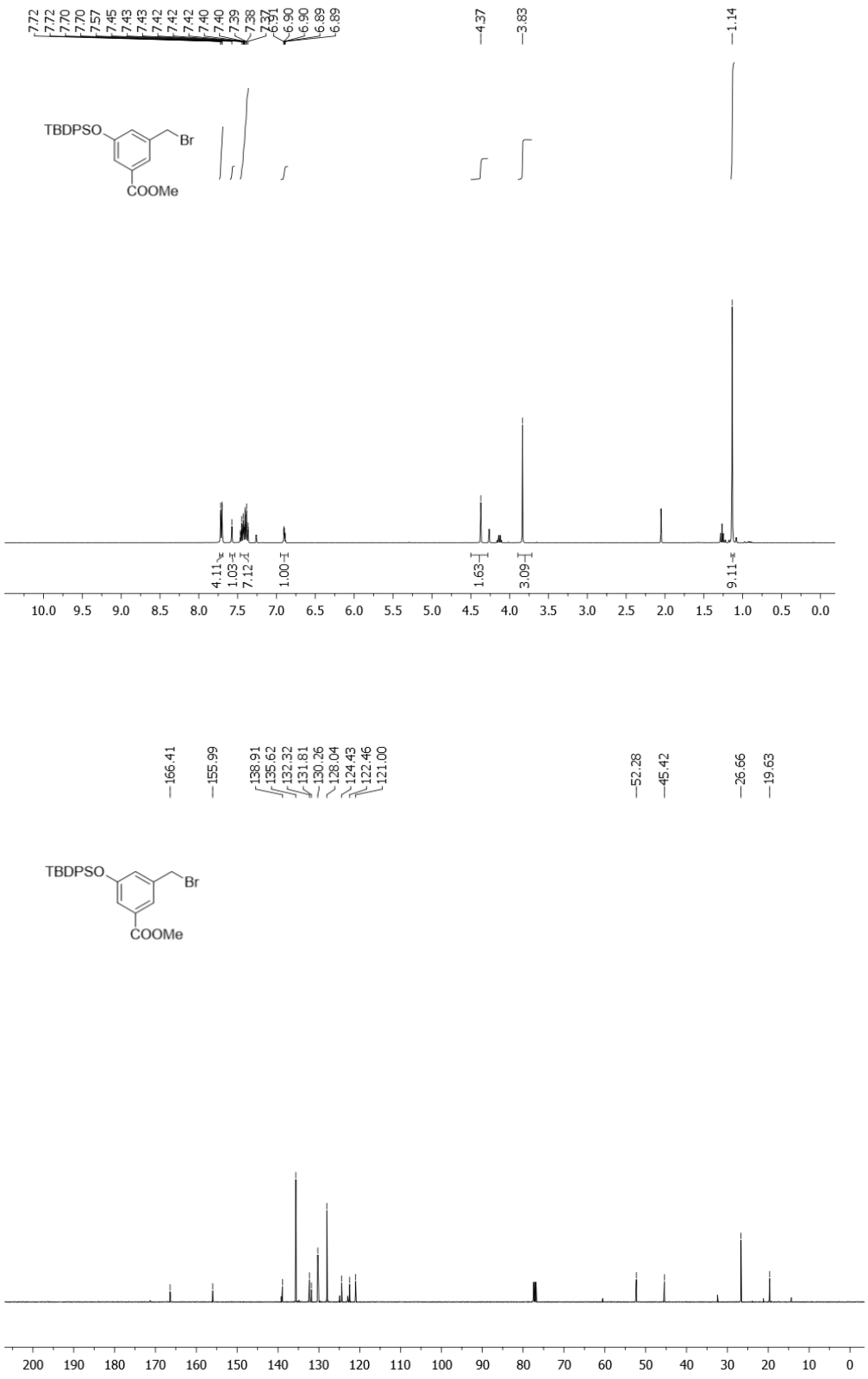


3-(Aminomethyl)-5-hydroxybenzoic acid (53)

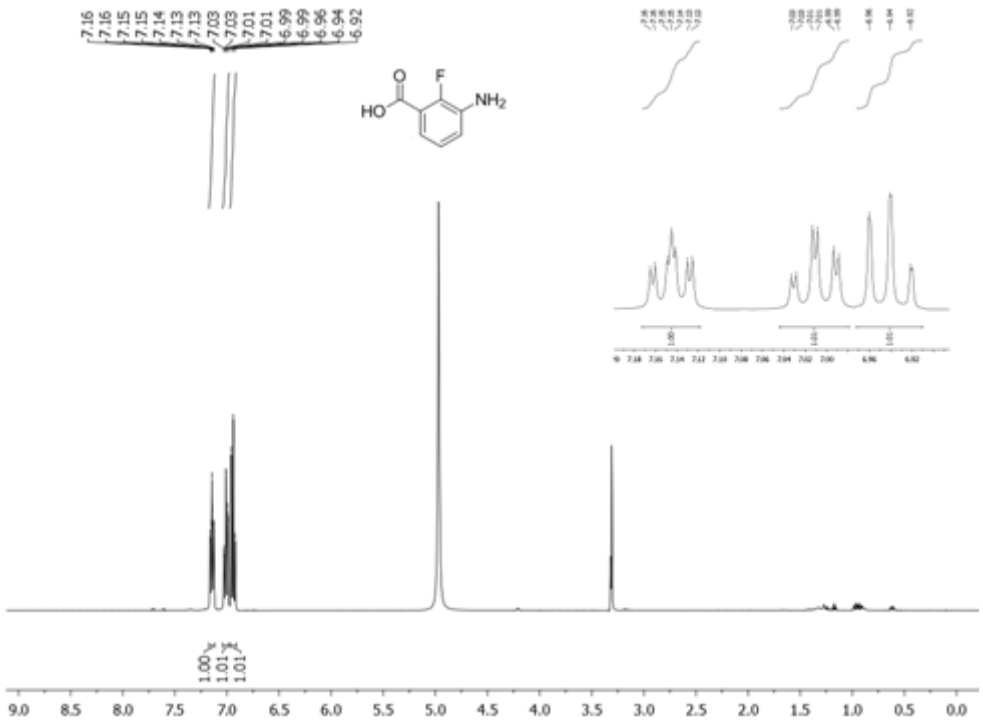


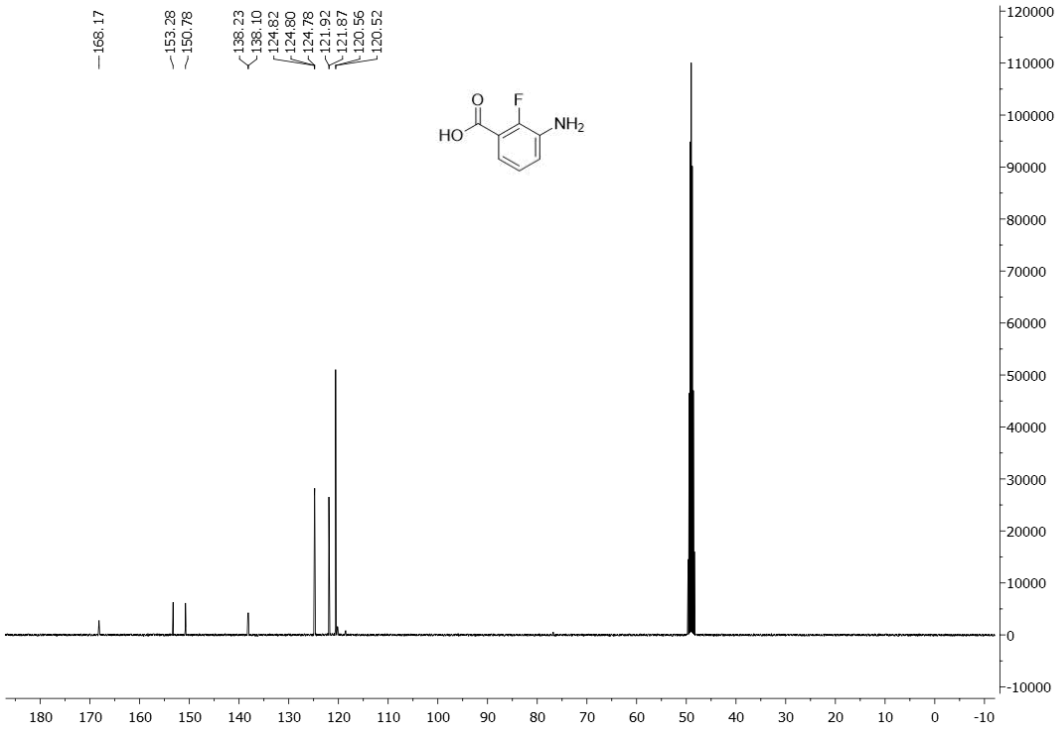


Methyl 3-(bromomethyl)-5-(*tert*-butyldiphenylsilyloxy)benzoate (50)

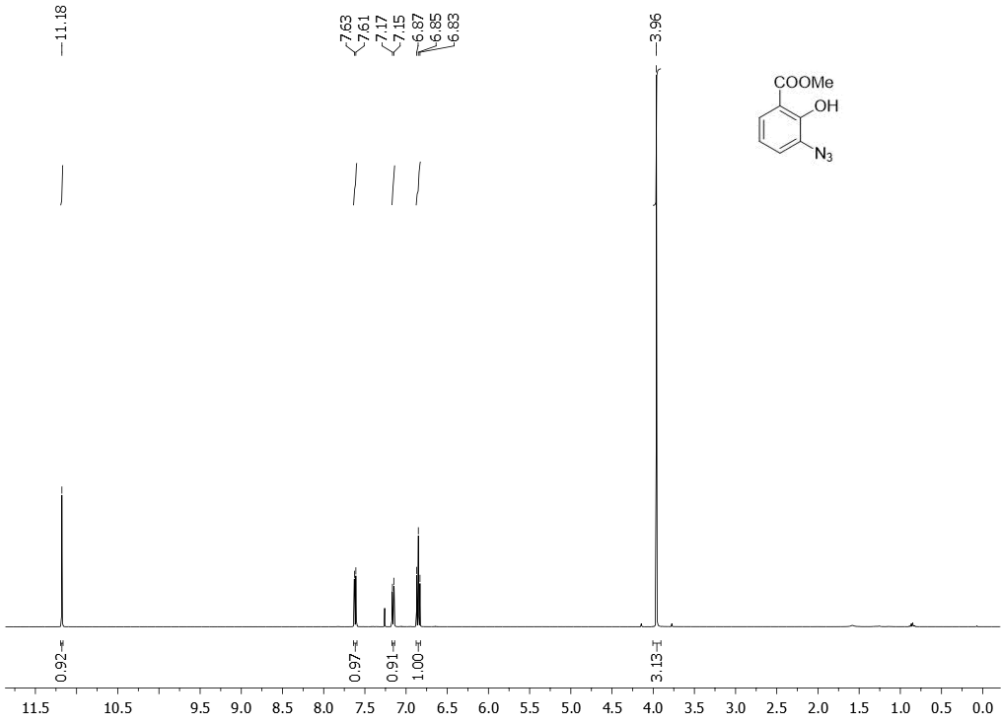


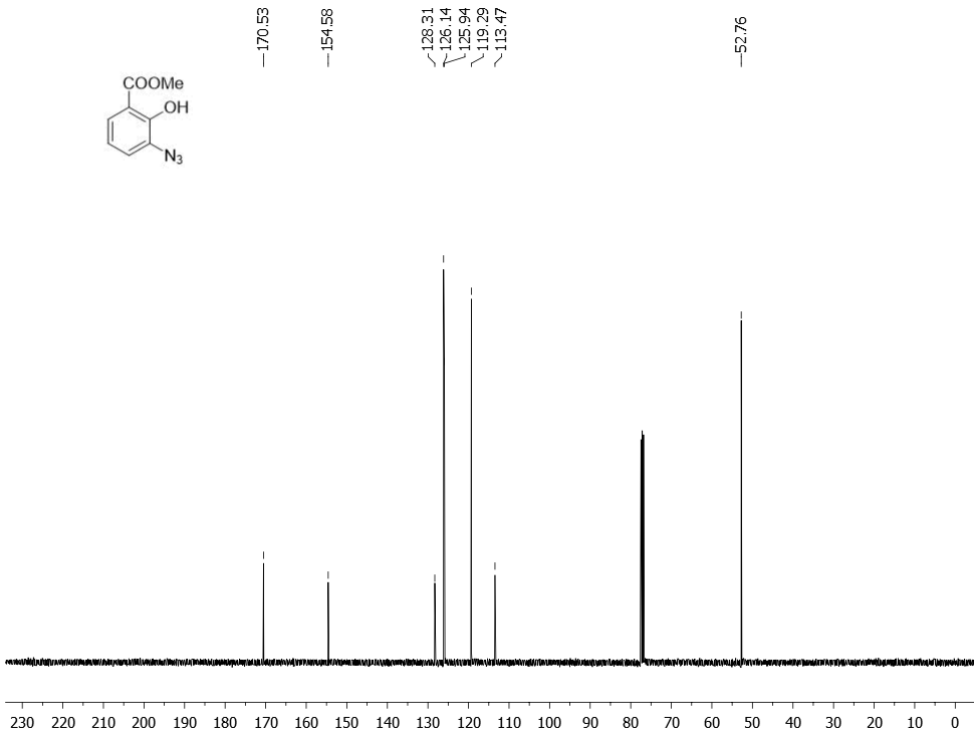
3-Amino-2-fluorobenzoic Acid (55)



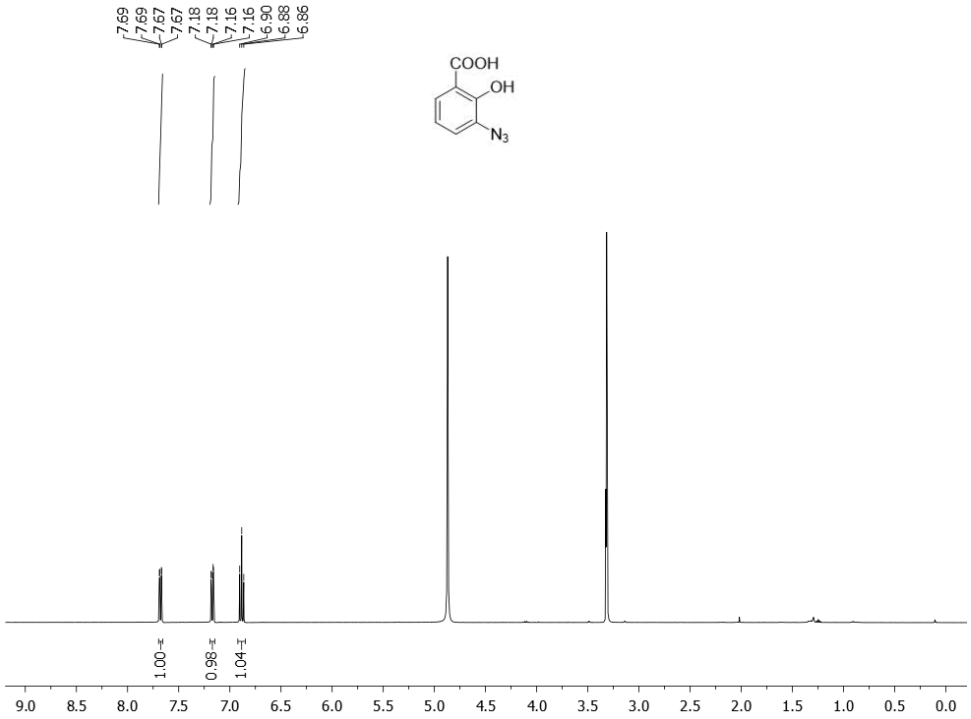


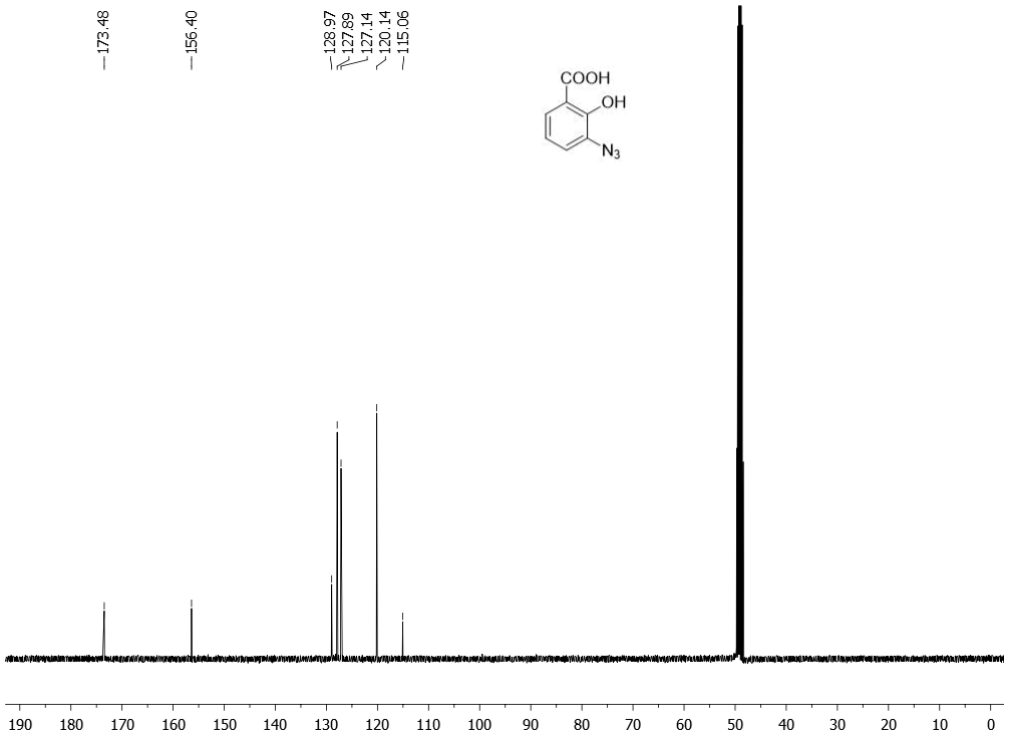
Methyl 3-azido-2-hydroxybenzoate (62)



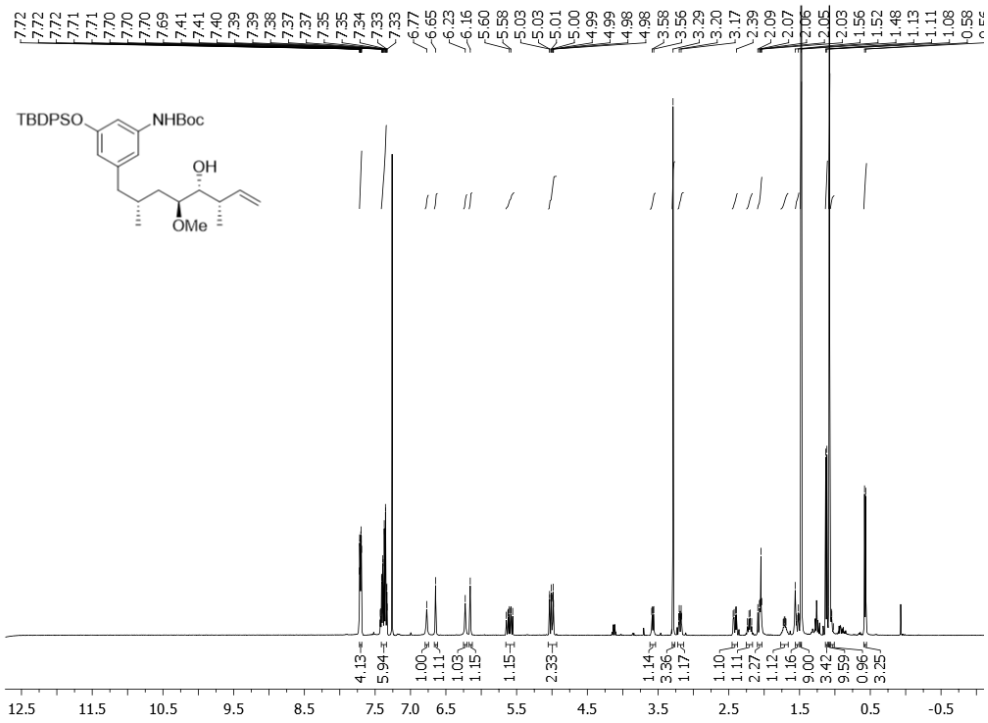


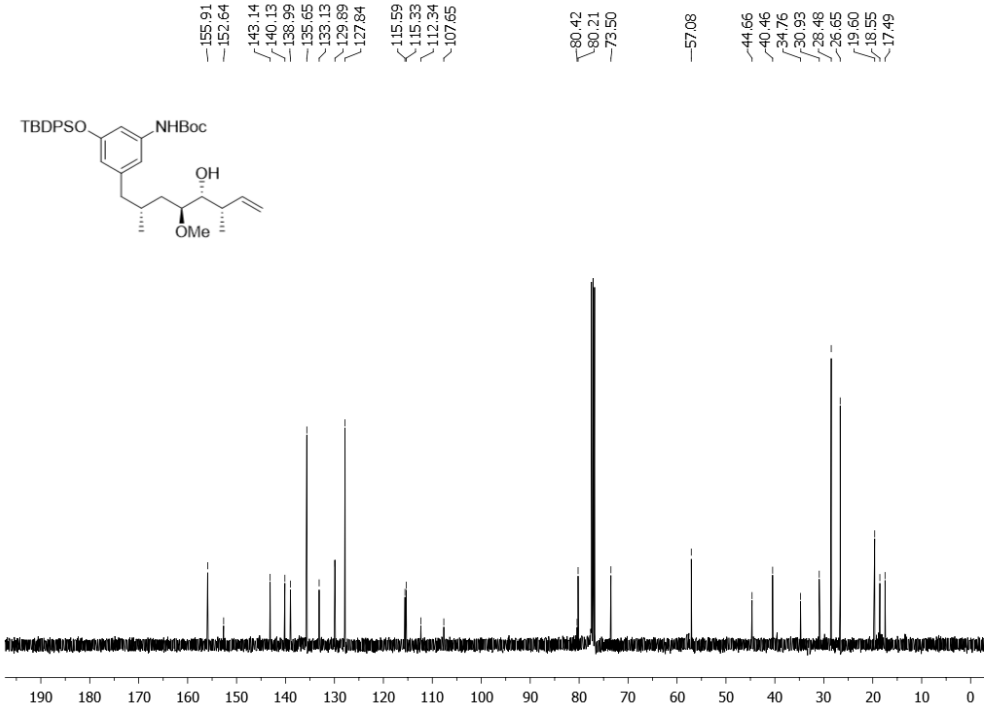
3-Azido-2-hydroxybenzoic acid (63)



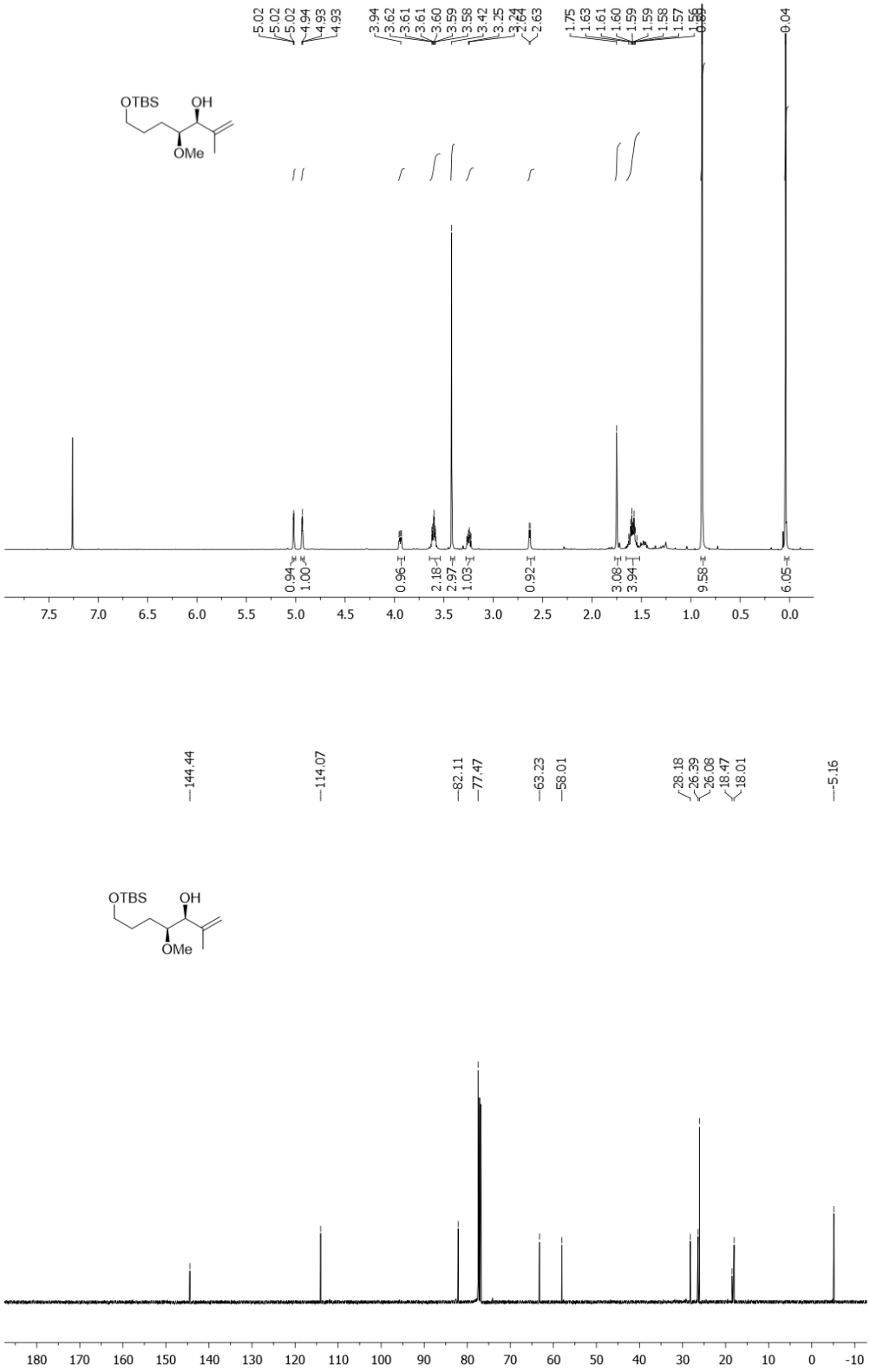


(3*S*,4*R*,5*S*,7*R*)-8-[3-(*tert*-Butoxycarbonylamino)-5-(*tert*-butyldiphenylsiloxy)-phenyl]-4-hydroxy-5-methoxy-3,7-dimethyl-oct-2-en (148)

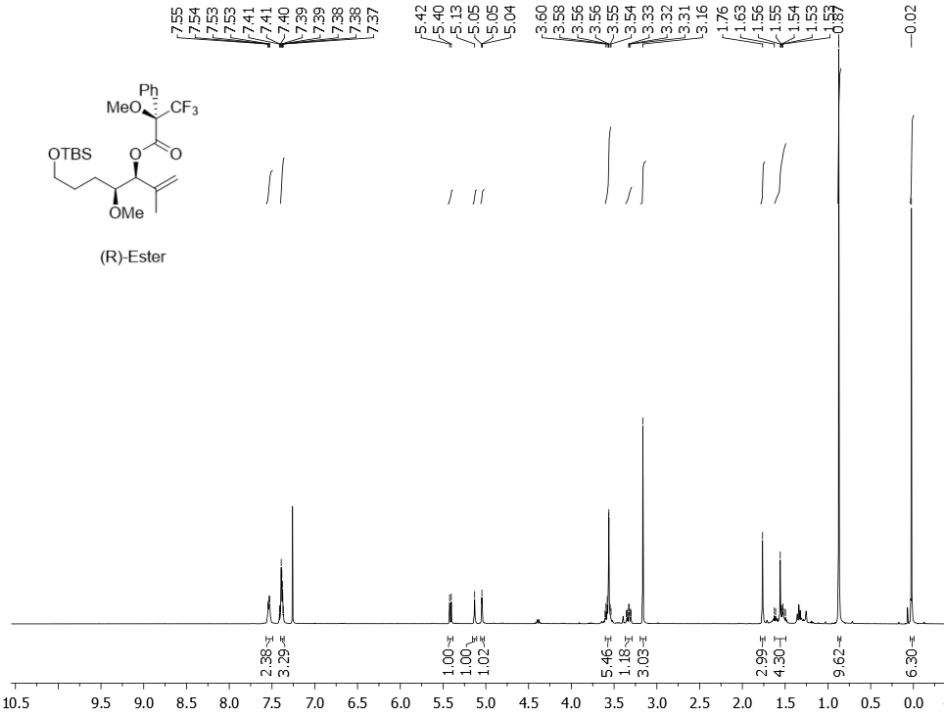




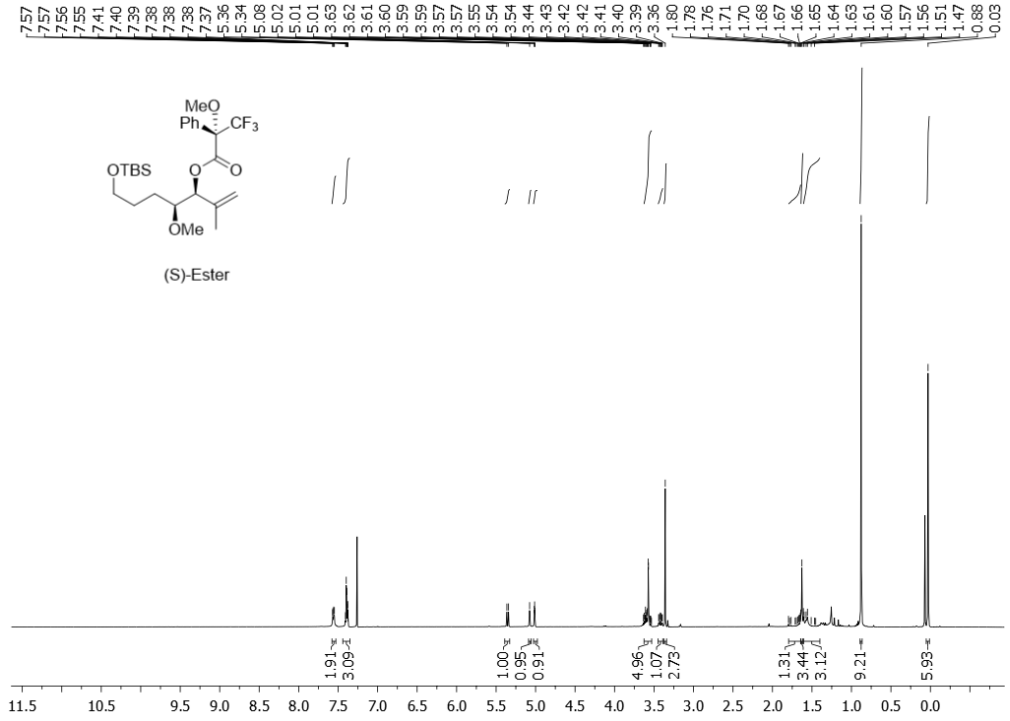
(3*S*,4*S*)-7-(*tert*-Butyldimethylsilyloxy)-4-methoxy-2-methylhept-1-en-3-ol (149)



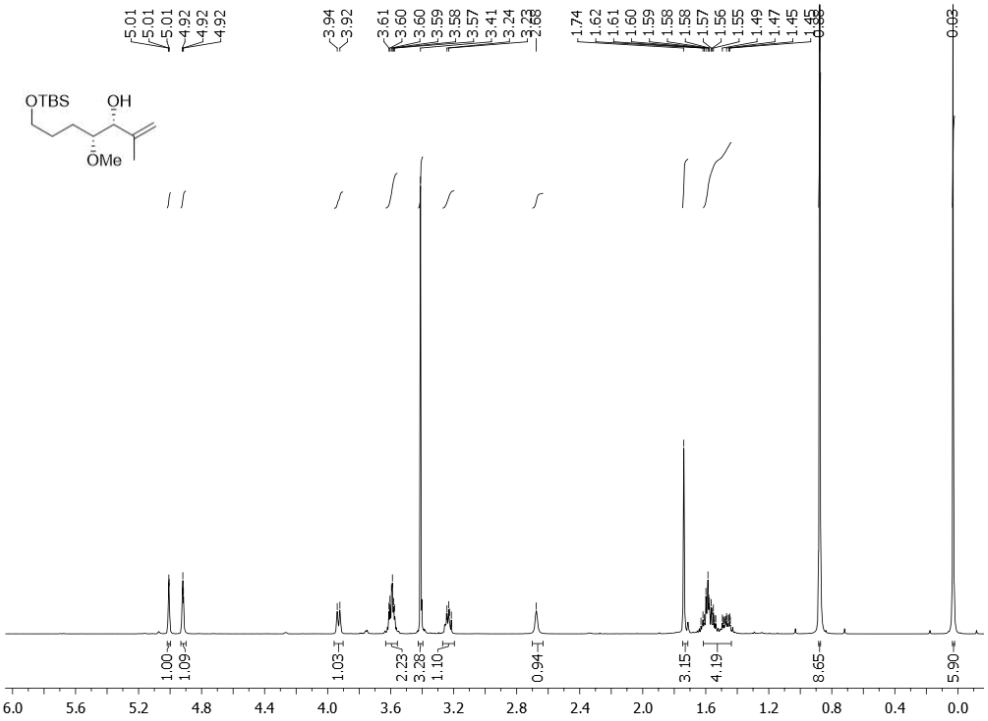
(*R*)-Mosher Ester (180a)

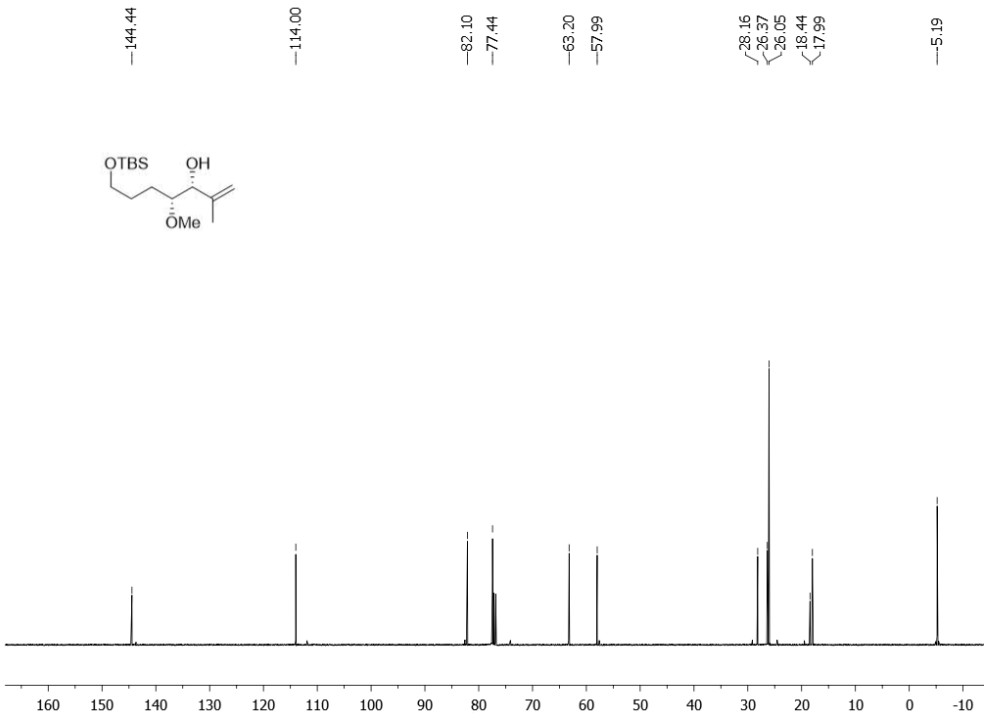


(*S*)-Mosher Ester (180b)

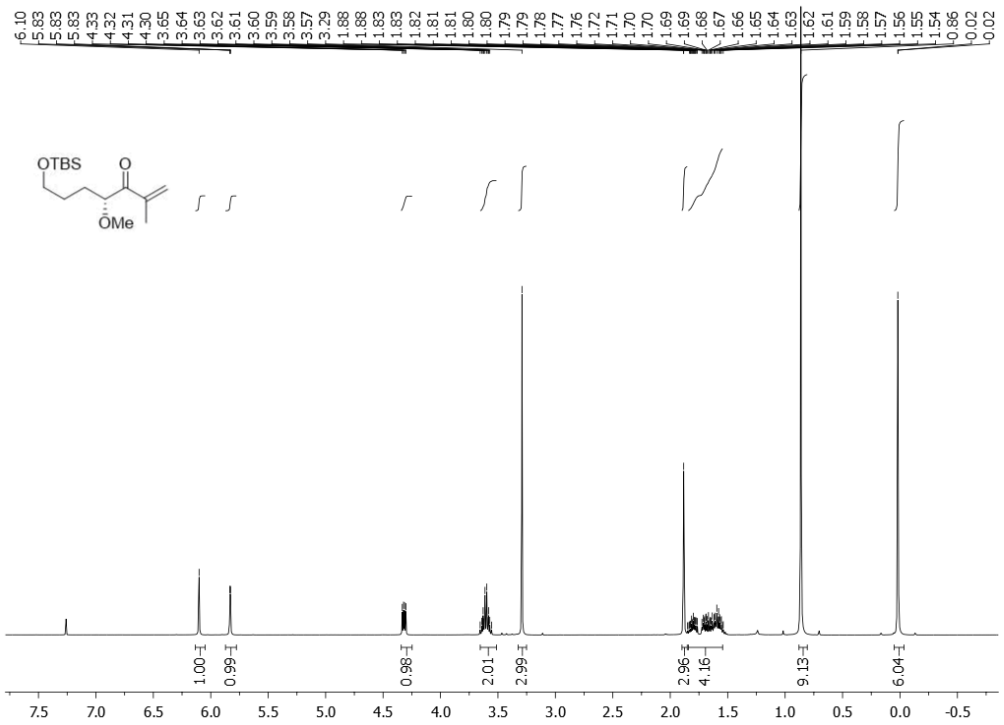


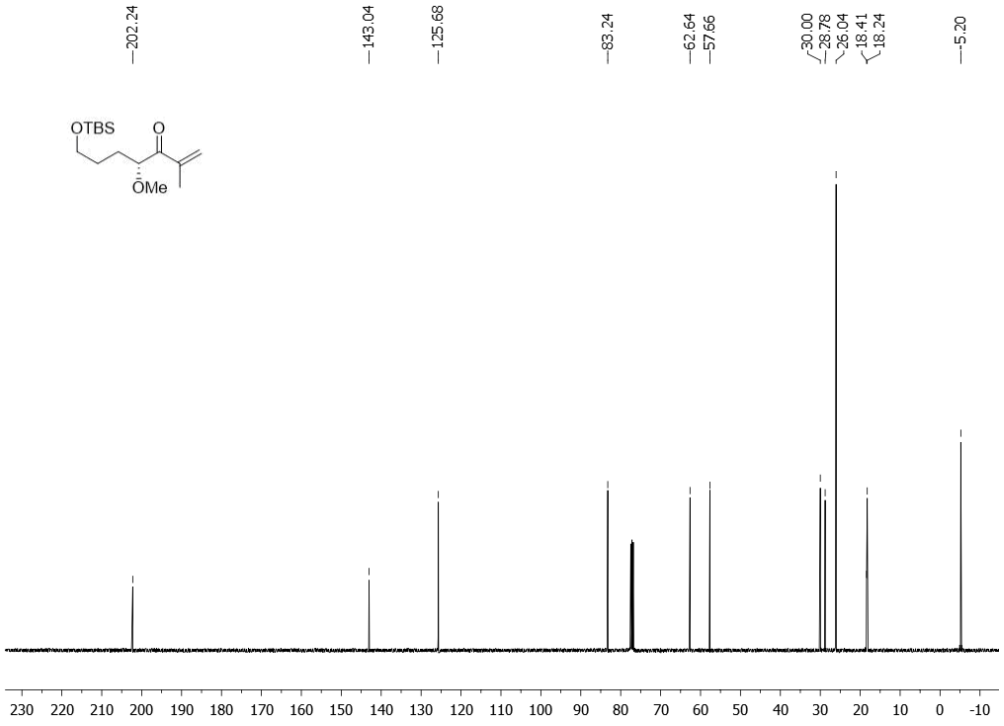
(3*R*,4*R*)-7-(*tert*-Butyldimethylsilyloxy)-4-methoxy-2-methylhept-1-en-3-ol (150b)



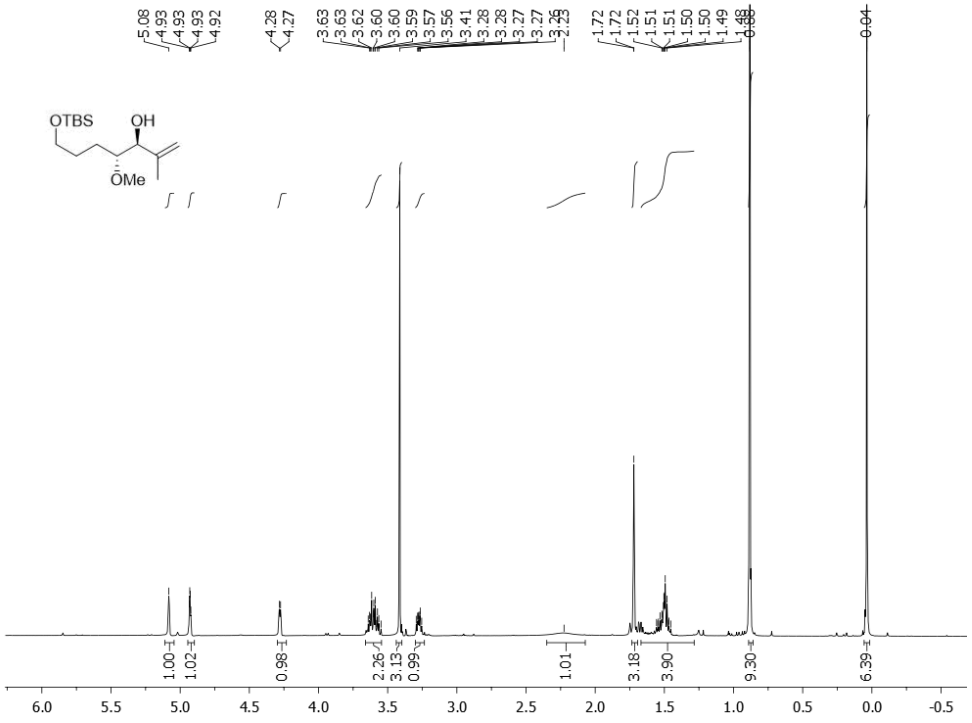


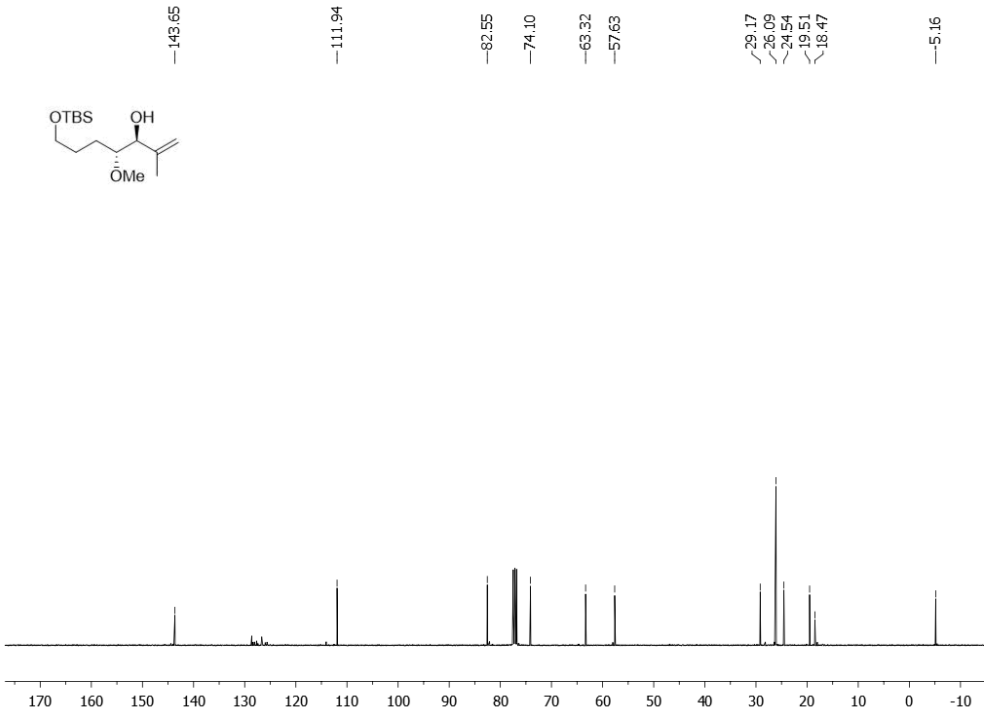
(*R*)-7-(*tert*-Butyldimethylsilyloxy)-4-methoxy-2-methylhept-1-en-3-one (183)



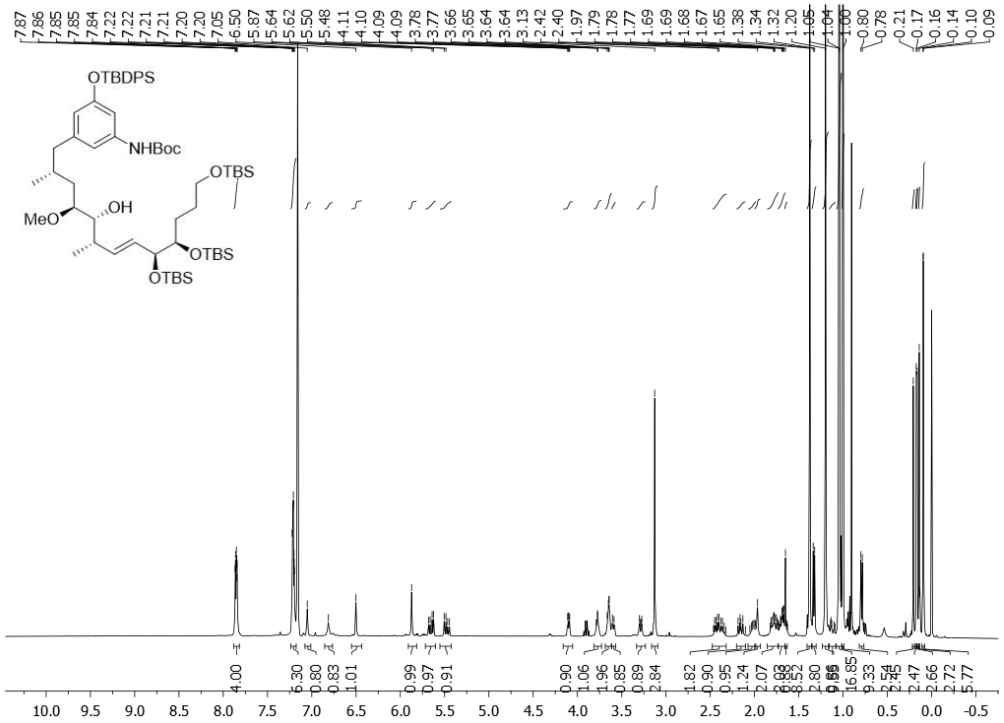


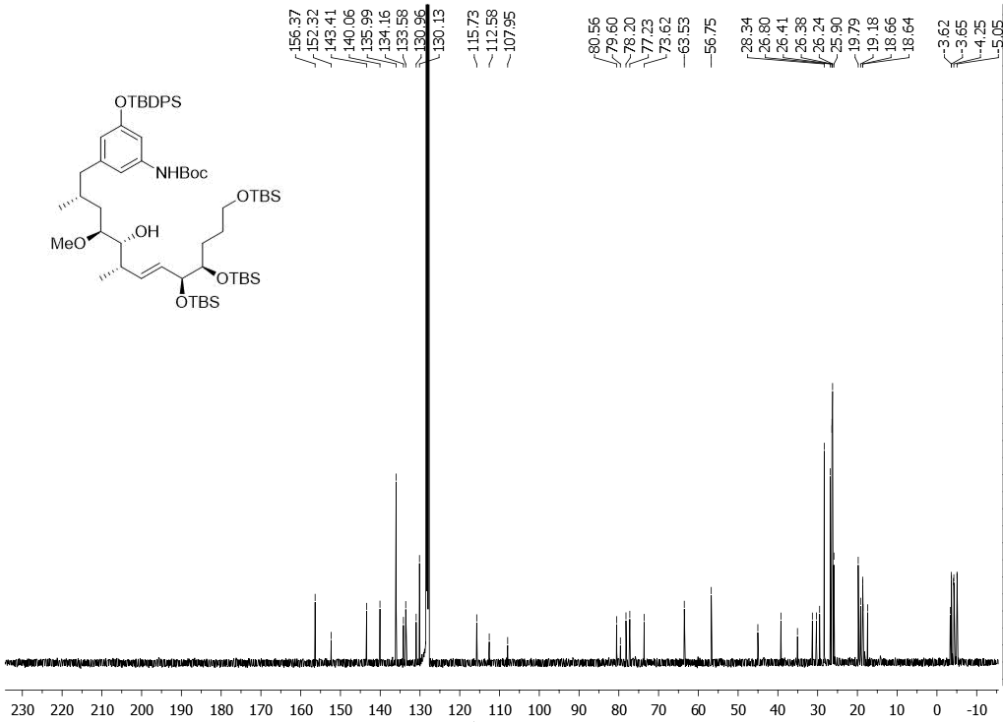
(3*S*,4*R*)-7-(*tert*-Butyldimethylsilyloxy)-4-methoxy-2-methylhept-1-en-3-ol (150a)



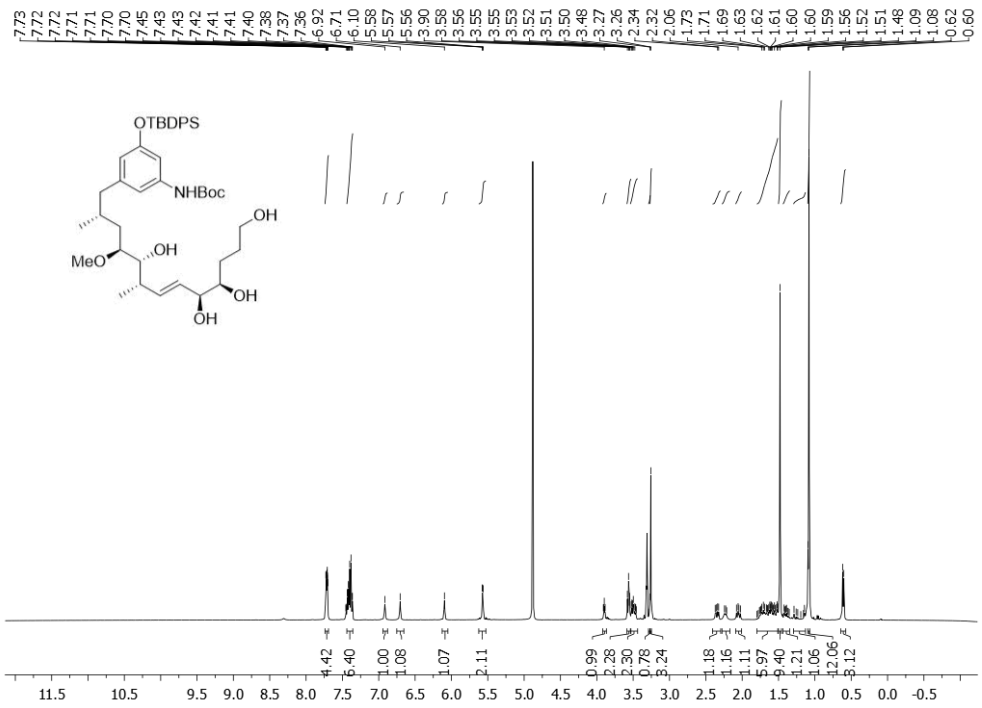


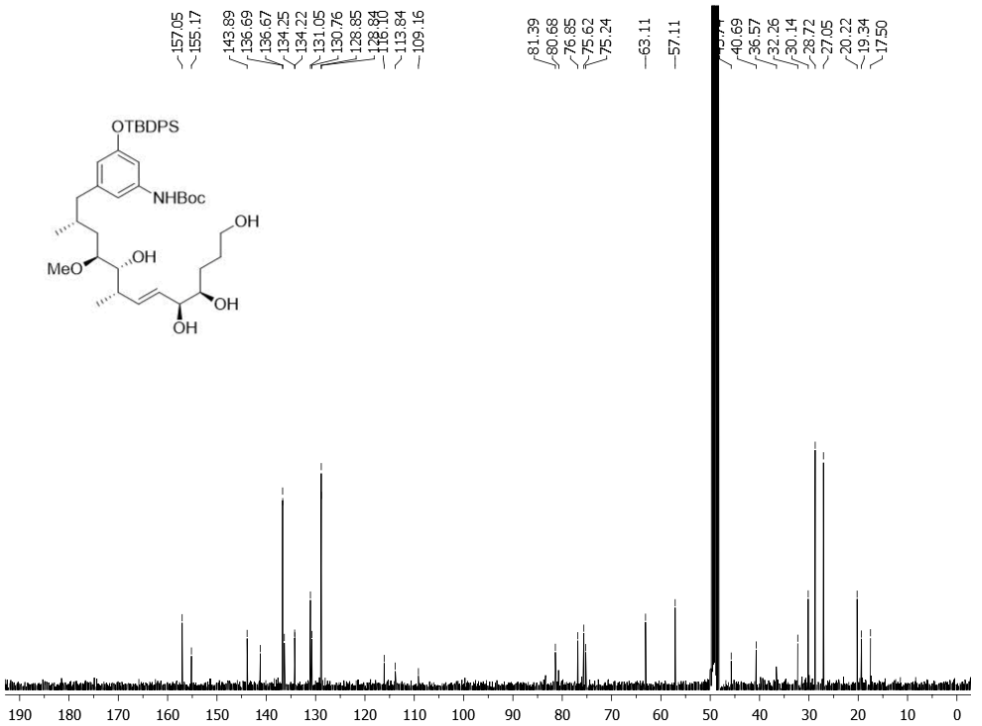
Silylated polyol 211



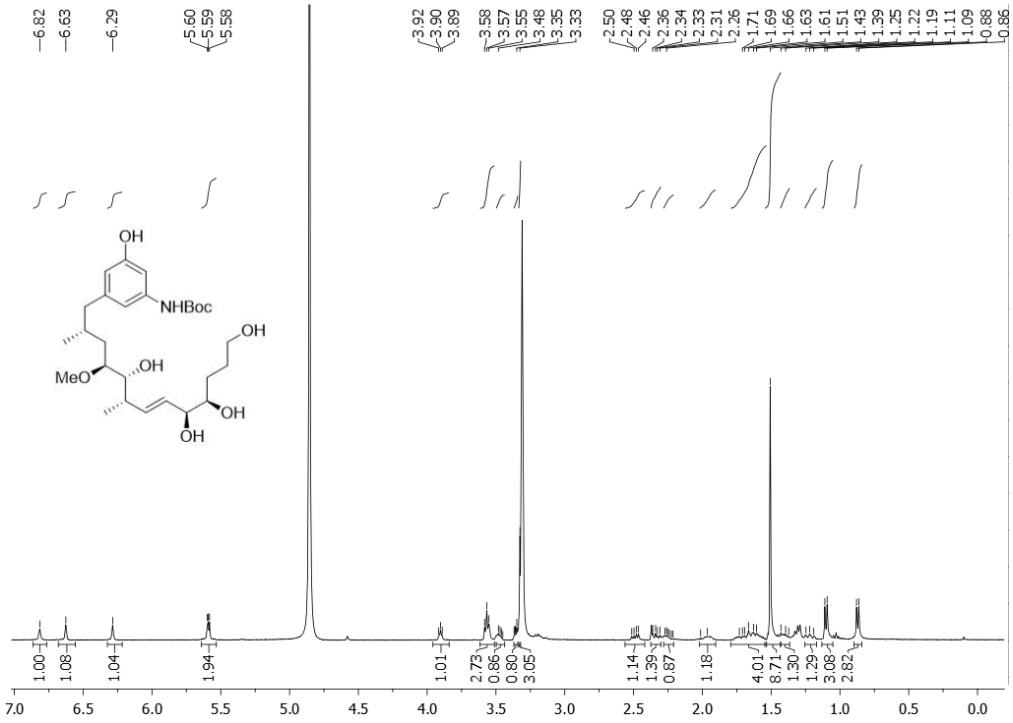


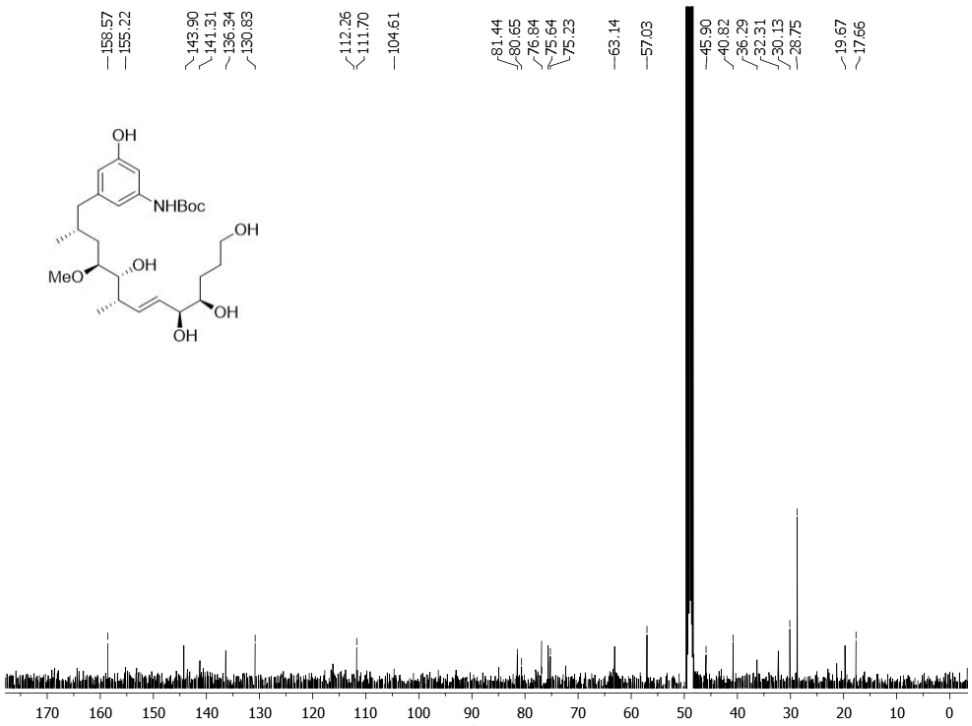
TBS-deprotected polyol 197



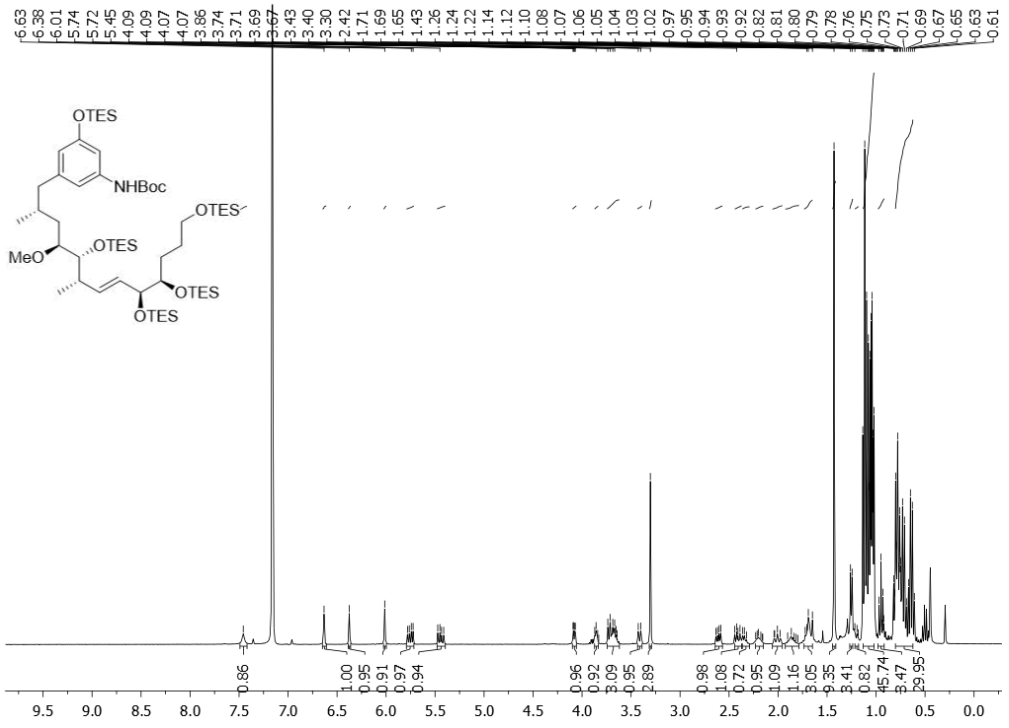


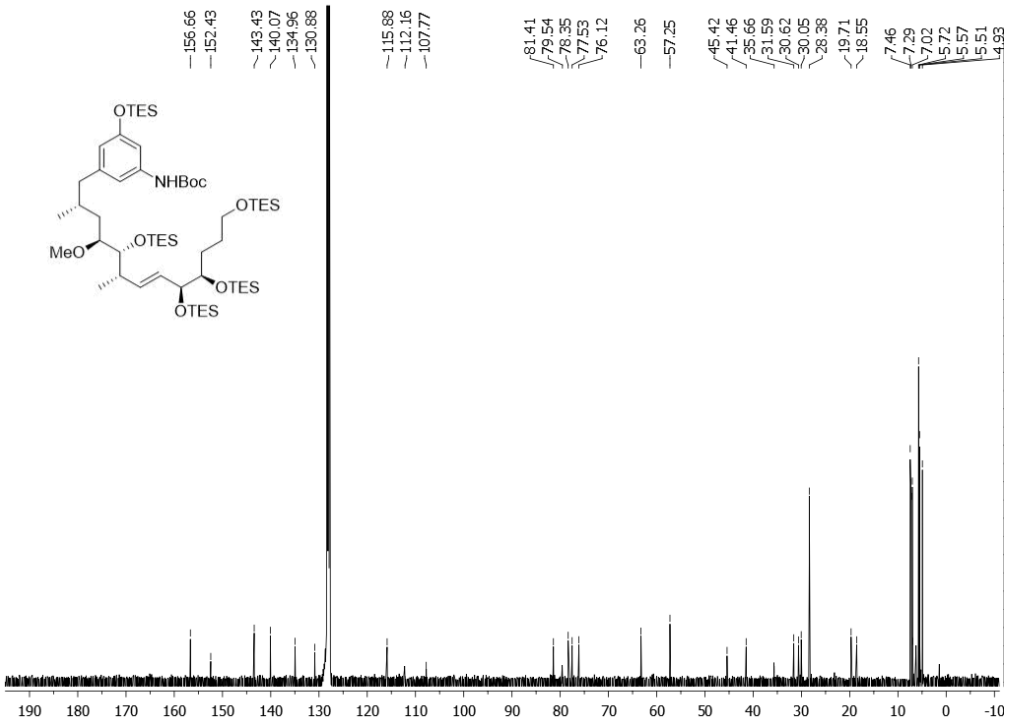
Polyol 198


6.82
6.63
6.29
5.60
5.59
5.58
3.92
3.90
3.89
3.58
3.57
3.55
3.48
3.35
3.33
2.50
2.48
2.46
2.36
2.34
2.33
2.31
2.26
1.71
1.69
1.66
1.63
1.61
1.51
1.43
1.39
1.25
1.22
1.19
1.11
1.09
0.88
0.86
OH
NHBoc
OH
MeO
OH
OH
OH
1.00
1.08
1.04
1.94
1.01
2.73
0.86
0.80
3.05
1.14
1.39
0.87
1.18
4.01
8.71
1.30
1.29
3.08
2.82
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

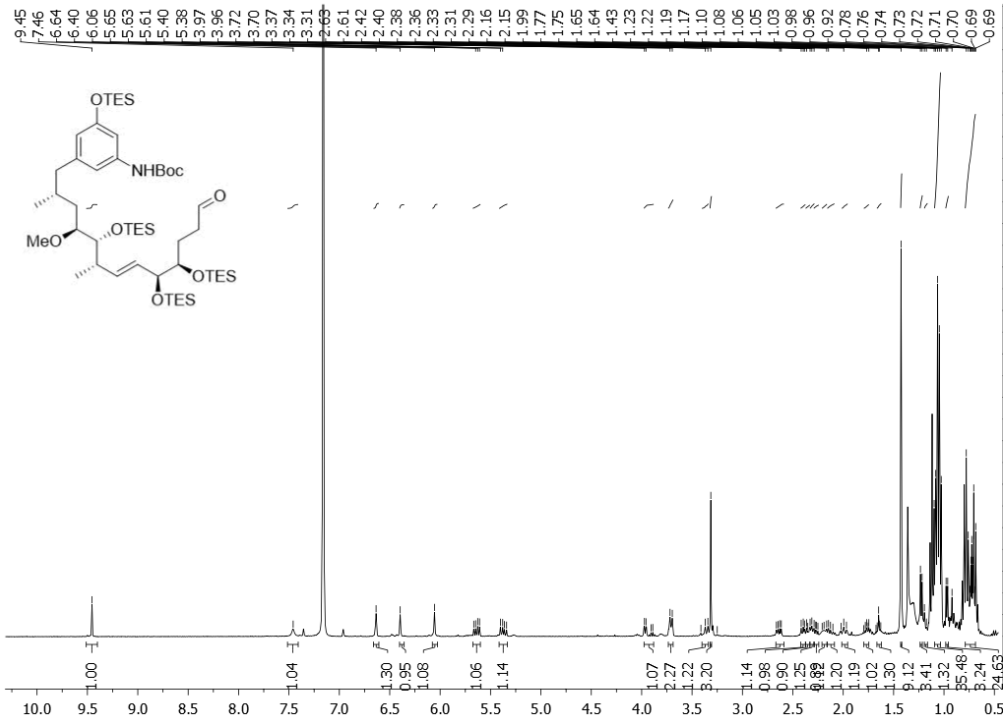

158.57
155.22
143.90
141.31
136.34
130.83
112.26
111.70
104.61
81.44
80.65
76.84
75.64
75.23
63.14
57.03
45.90
40.82
36.29
32.31
30.13
28.75
19.67
17.66
OH
NHBoc
OH
MeO
OH
OH
OH
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

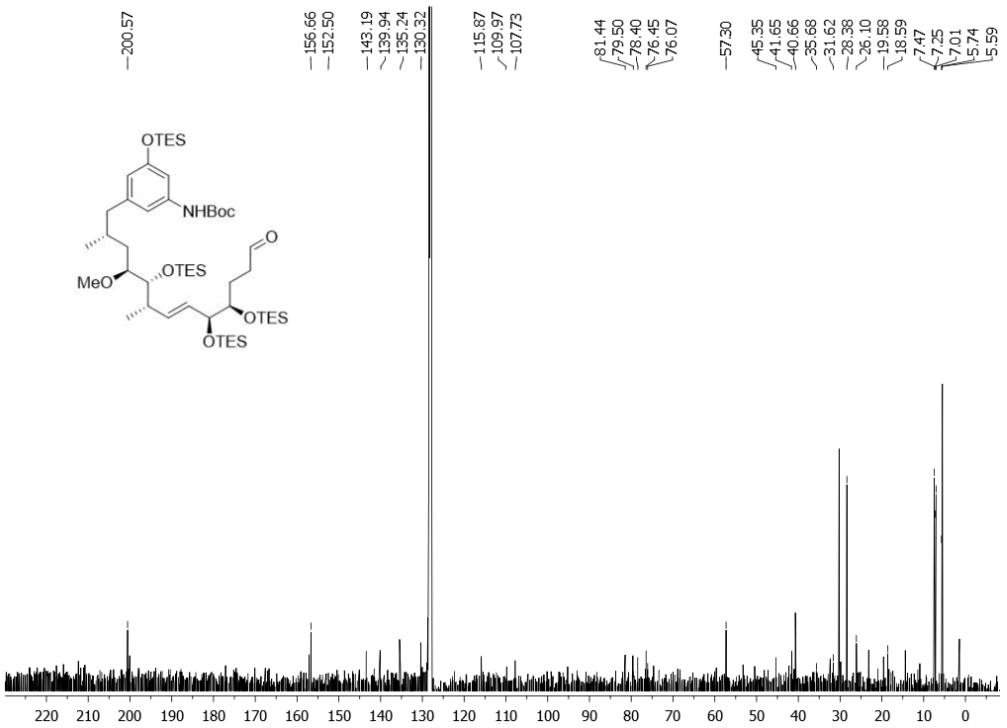
TES-protected polyol 199



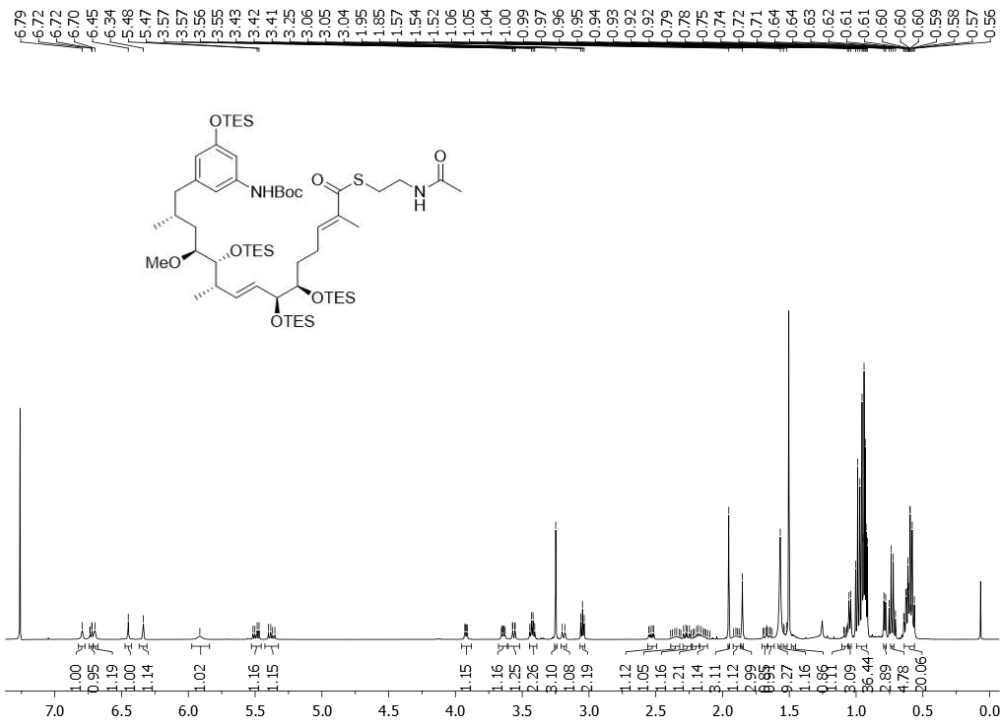


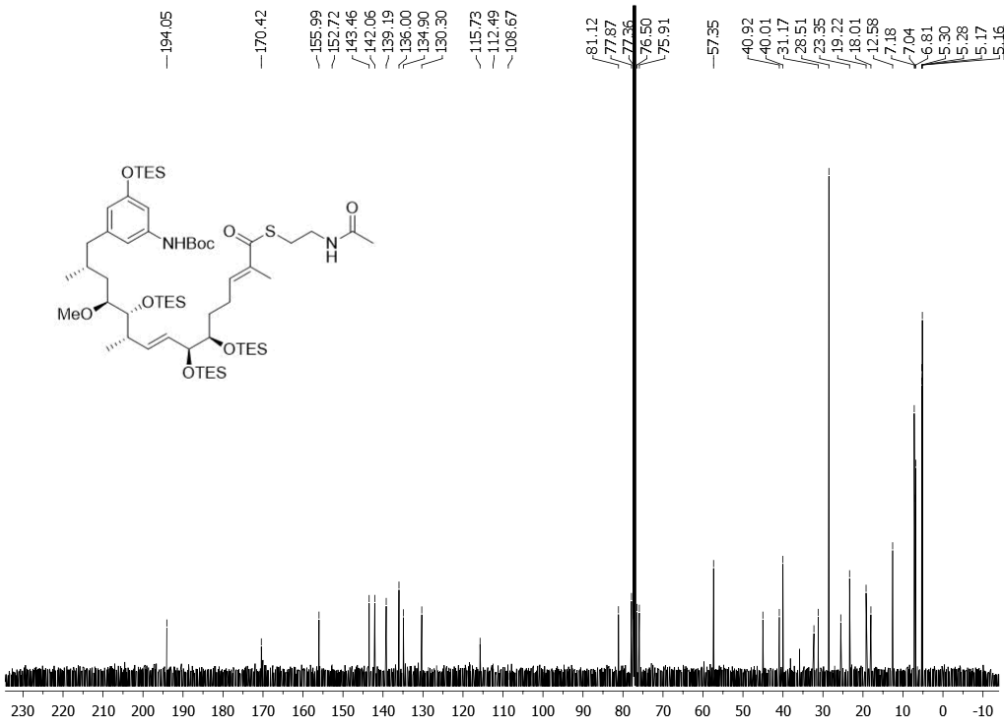
Silylated aldehyde 200



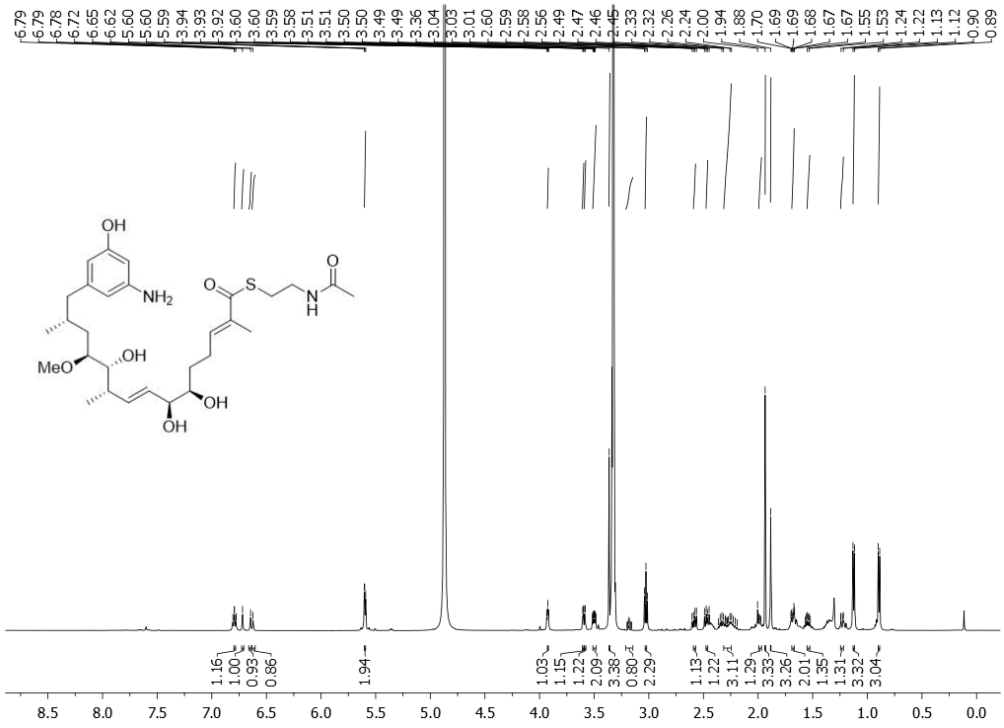


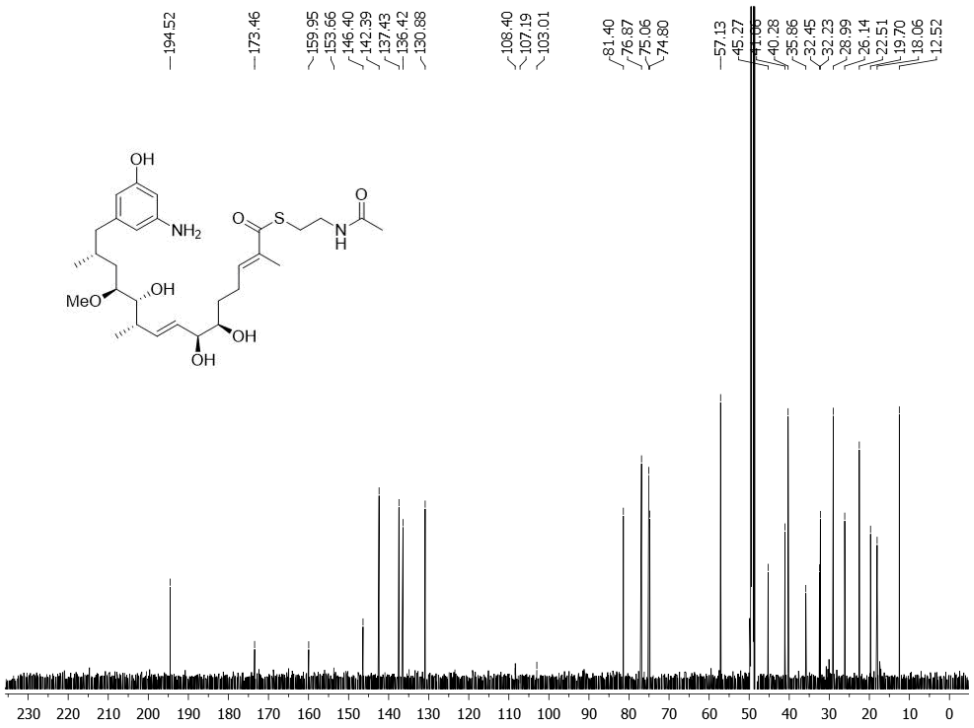
Silylated SNAc ester 201





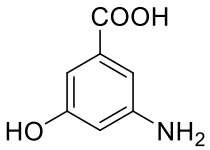
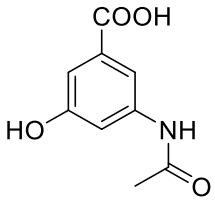
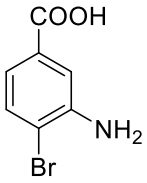
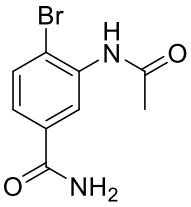
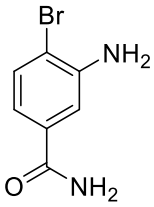
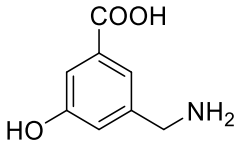
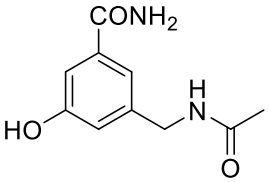
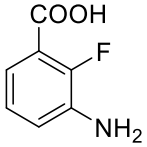
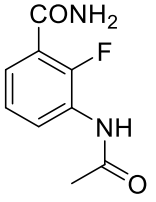
SNAc ester 137

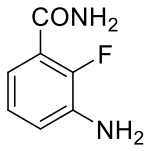
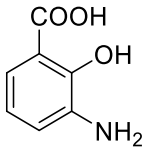
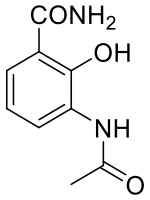
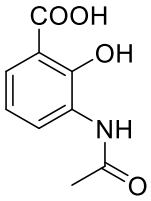
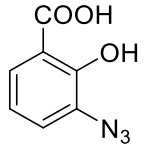
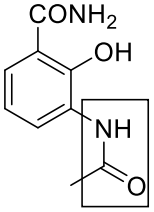
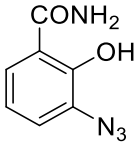
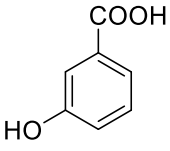
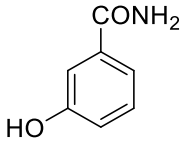


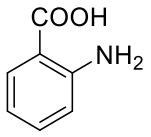
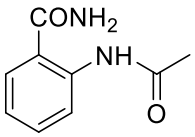
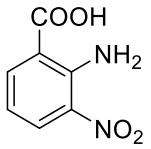
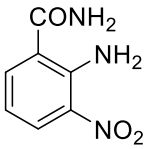
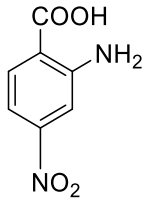
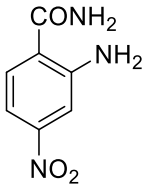
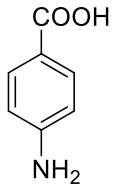
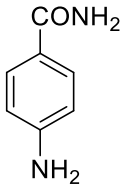
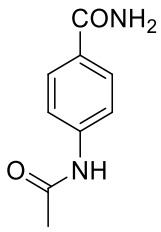
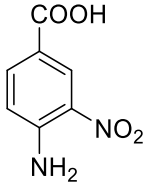
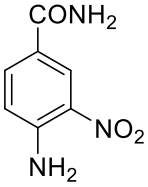


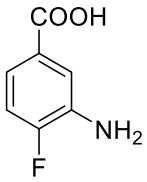
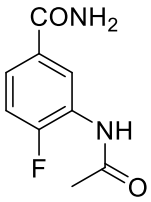
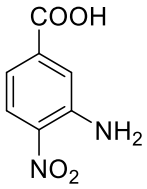
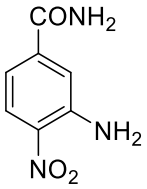
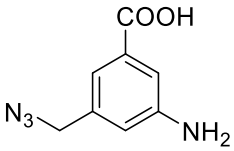
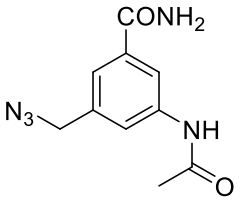
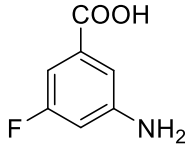
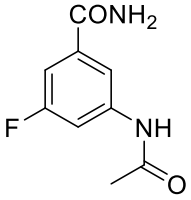
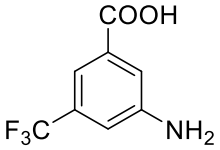
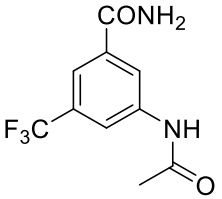
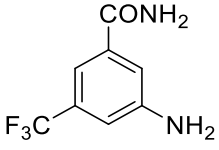
4.2 MS Data of Detoxification Products

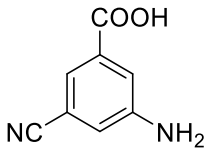
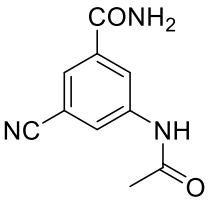
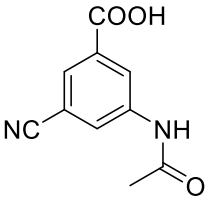
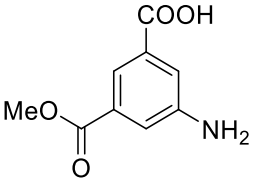
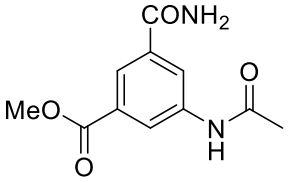
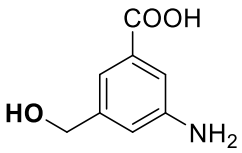
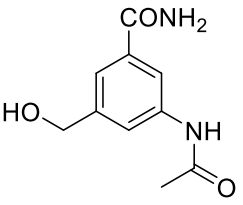
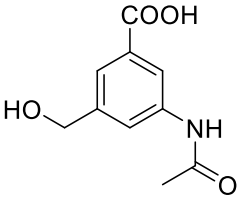
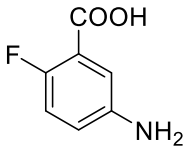
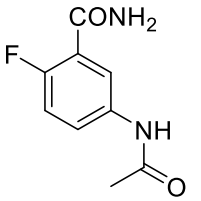
In most fermentations formation of "detoxification" products occurred. Either the (amino)benzoic acids were *N*-acylated or the carboxylic acid was transformed into the amide.

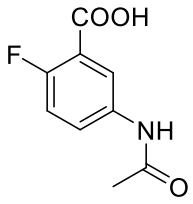
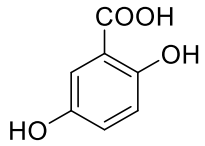
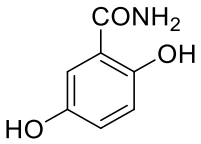
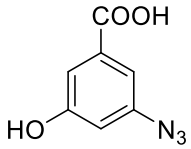
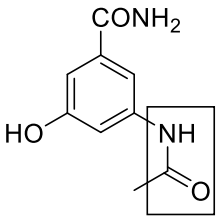
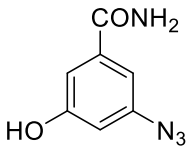
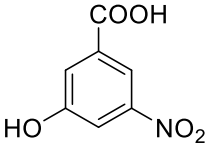
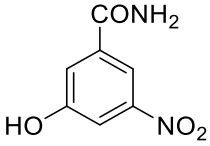
Mutasynthons	Detoxification Products	HRMS data
 **7**		$[M+H]^+$ calcd for $[C_9H_{10}NO_4]^+$: 196.0610 found: 196.0612 t_R = 1.23 min
 **41**		$[M+H]^+$ calcd for $[C_9H_{10}BrN_2O_2]^+$: 256.9926 found: 256.9932 t_R = 1.9 min
		$[M+H]^+$ calcd for $[C_7H_8BrN_2O]^+$: 214.9820 found: 214.9820 t_R = 2.1 min
 **53**		$[M+H]^+$ calcd for $[C_{10}H_{13}N_2O_3]^+$: 209.0926 found: 209.0929 t_R = 1.35 min
 **55**		$[M+H]^+$ calcd for $[C_9H_{10}FN_2O_2]^+$: 197.0726 found: 197.0733 t_R = 1.83 min

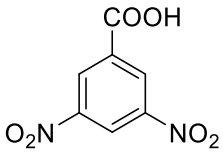
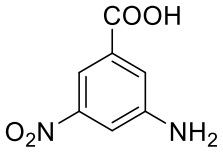
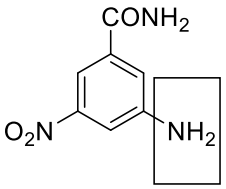
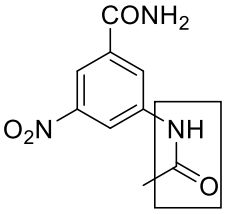
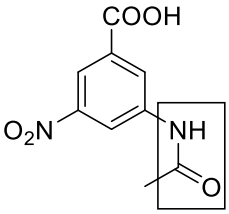
		$[M+H]^+$ calcd for $[C_7H_8FN_2O]^+$: 155.0621 found: 155.0629 t_R = 1.57 min
 **61**		$[M-H]^-$ calcd for $[C_9H_9N_2O_3]^-$: 193.0619 found: 193.0605 t_R = 1.9 min
		$[M-H]^-$ calcd for $[C_9H_8NO_4]^-$: 194.0459 found: 194.0454 t_R = 2.1 min
 **63**		$[M-H]^-$ calcd for $[C_9H_9N_2O_3]^-$: 193.0619 found: 193.0612 t_R = 1.9 min
		$[M+H]^+$ calcd for $[C_7H_7N_4O_2]^+$: 179.0569 found: 179.0560 $[M-H]^-$ calcd for $[C_7H_5N_4O_2]^-$: 177.0418 found: 177.0410 t_R = 1.92 min
 **64**		$[M+H]^+$ calcd for $[C_7H_8NO_2]^+$: 138.0555 found: 138.0565 t_R = 1.62 min

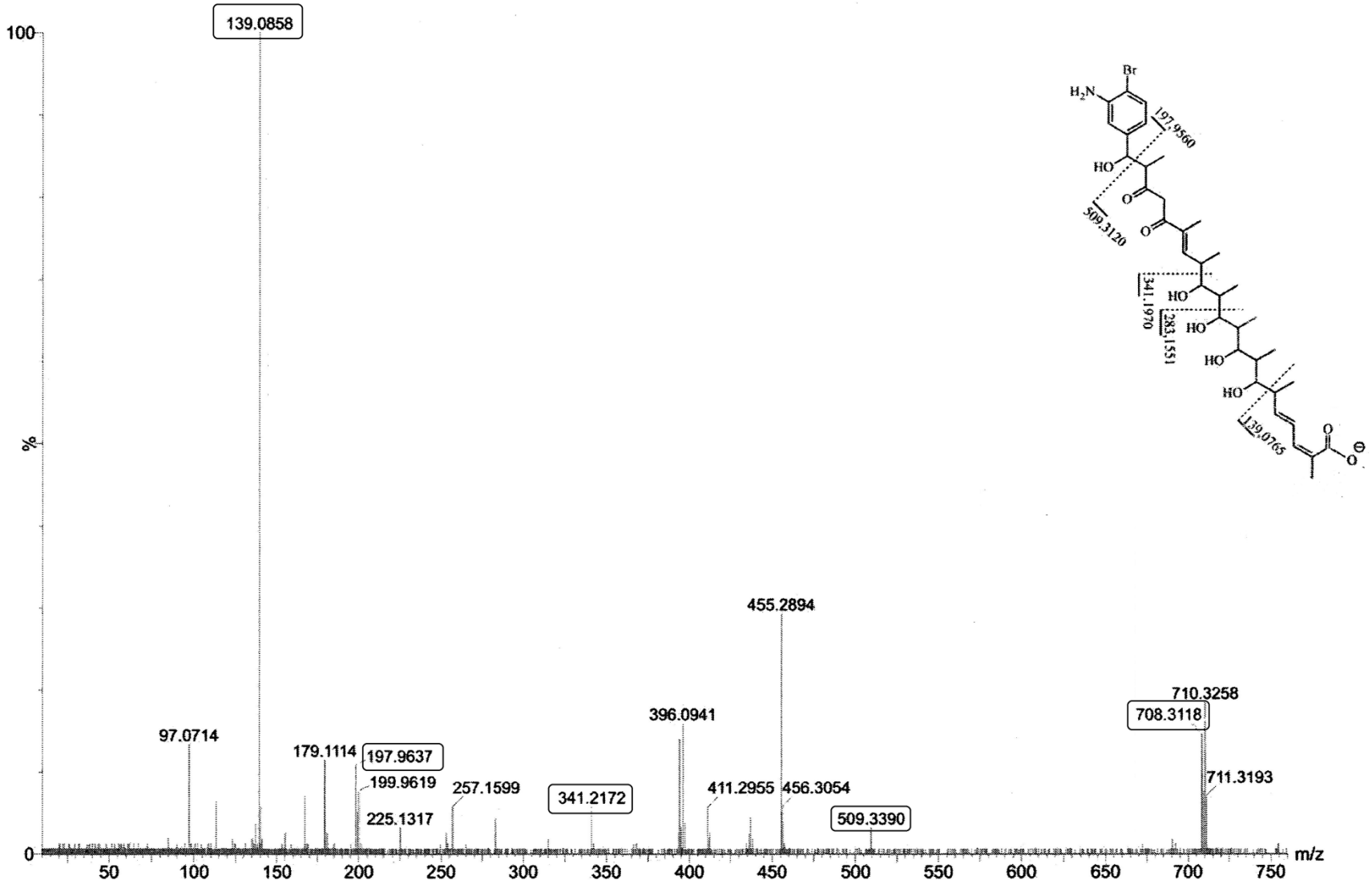
 **70**		$[M+H]^+$ calcd for $[C_9H_{11}N_2O_3]^+$: 179.0821 found: 179.0825 t_R = 1.26 min
 **71**		$[M+H]^+$ calcd for $[C_7H_8N_3O_3]^+$: 182.0566 found: 182.0570 t_R = 2.17 min
 **72**		$[M+H]^+$ calcd for $[C_7H_8N_3O_3]^+$: 182.0566 found: 182.0567 t_R = 1.85 min
 **73**		$[M+H]^+$ calcd for $[C_7H_9N_2O]^+$: 137.0715 found: 137.0716 t_R = 0.35 min
		$[M+H]^+$ calcd for $[C_9H_{11}N_2O_2]^+$: 179.0821 found: 179.0821 t_R = 0.93 min
 **74**		$[M+H]^+$ calcd for $[C_7H_8N_3O_3]^+$: 182.0566 found: 182.0565 t_R = 1.84 min

 **75**		$[M+H]^+$ calcd for $[C_9H_{10}FN_2O_2]^+$: 197.0726 found: 197.0723 t_R = 1.85 min
 **76**		$[M+H]^+$ calcd for $[C_7H_8N_3O_3]^+$: 182.0566 found: 182.0563 t_R = 1.83 min
 **77**		$[M+H]^+$ calcd for $[C_{10}H_{12}N_5O_2]^+$: 234.0991 found: 234.0990 t_R = 2.0 min
 **78**		$[M+H]^+$ calcd for $[C_9H_{10}FN_2O_2]^+$: 197.0726 found: 197.0722 t_R = 2.0 min
 **79**		$[M+H]^+$ calcd for $[C_{10}H_{10}F_3N_2O_2]^+$: 247.0694 found: 247.0688 t_R = 2.4 min
		$[M+H]^+$ calcd for $[C_8H_8F_3N_2O]^+$: 205.0589 found: 205.0592 t_R = 2.2 min

 **80**		$[M+H]^+$ calcd for $[C_{10}H_{10}N_3O_2]^+$: 204.0773 found: 204.0770 t_R = 1.9 min
		$[M+H]^+$ calcd for $[C_{10}H_9N_2O_3]^+$: 205.0613 found: 205.0610 t_R = 2.0 min
 **81**		$[M+H]^+$ calcd for $[C_{11}H_{12}N_2O_4]^+$: 237.0875 found: 237.0881 t_R = 2.1 min
 **82**		$[M+H]^+$ calcd for $[C_{10}H_{13}N_2O_3]^+$: 209.0926 found: 209.0921 t_R = 1.05 min
		$[M+H]^+$ calcd for $[C_{10}H_{12}NO_4]^+$: 210.0766 found: 210.0775 t_R = 1.12 min
 **83**		$[M+H]^+$ calcd for $[C_9H_{10}FN_2O_2]^+$: 197.0726 found: 197.0721 t_R = 1.8 min

		$[M+H]^+$ calcd for $[C_9H_9FNO_3]^+$: 198.0566 found: 198.0570 t_R = 2.0 min
 **86**		$[M+H]^+$ calcd for $[C_7H_8NO_3]^+$: 154.0504 found: 154.0508 t_R = 1.21 min
 **94**		$[M+H]^+$ calcd for $[C_9H_{11}N_2O_3]^+$: 195.0770 found: 195.0768 $[M-H]^-$ calcd for $[C_9H_9N_2O_3]^-$: 193.0619 found: 193.0610 t_R = 1.6 min
		$[M+H]^+$ calcd for $[C_7H_7N_4O_2]^+$: 179.0569 found: 179.0559 t_R = 2.1 min
 **95**		$[M+H]^+$ calcd for $[C_7H_7N_2O_4]^+$: 183.0406 found: 183.0405 t_R = 2.0 min

 **97**  **98**		$[M+H]^+$ calcd for $[C_7H_8N_3O_3]^+$: 182.0566 found: 182.0567 t_R = 1.8 min
		$[M+H]^+$ calcd for $[C_9H_{10}N_3O_4]^+$: 224.0671 found: 224.0670 t_R = 2.1 min
		$[M+H]^+$ calcd for $[C_9H_9N_2O_5]^+$: 225.0511 found: 225.0518 t_R = 2.4 min


100
%
0
139.0858
97.0714
179.1114
197.9637
199.9619
225.1317
257.1599
341.2172
396.0941
411.2955
455.2894
456.3054
509.3390
708.3118
710.3258
711.3193
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
m/z
Br
H2N
HO
O
197.9560
509.3120
341.1970
283.1551
139.0765

4.3 List of Abbreviations

$[\alpha]^{20}_{D}$	specific optical rotation
Å	angstrom
Ac	acetyl
ACP	acyl carrier protein
AHBA	3-amino-5-hydroxybenzoic acid
AminoDAHP	3,4-dideoxy-4-amino-D-*arabino*-heptulosinic acid 7-phosphate
AminoDHQ	5-deoxy-5-amino-3-dehydroquinic acid
AminoDHS	5-deoxy-5-amino-3-dehydroshikimic acid
Ar	aryl
AT	acyltransferase
ATP	adenosine triphosphate
b	NMR: broad
Boc	*tert*-butoxycarbonyl
t-Bu	*tert*-butyl
CBS	Corey-Bakshi-Shibata
c	concentration
ca	circa
calcd	calculated
$CHCl_3$	chloroform
CoA	coenzyme A
COSY	homonuclear correlation spectroscopy
CSA	camphorsulfonic acid
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
dest.	distilled
DET	diethyl tartrate
DH	dehydratase
DIPEA	*N*,*N*-diisopropylethylamine
DIBAL	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMDARSV	demethyl-deacetyl-rifamycin SV
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
DMRSV	27-*O*-demethylrifamycin SV
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
d.r.	diastereomeric ratio
EDC	*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide
ER	enoyl reductase
ESI	electrospray ionization
Et_3N	triethylamine
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
eq.	equivalent
g	gram
gdm	gene in the geldanamycin biosynthesis by *Streptomyces hygroscopicus*
Gdm	enzyme in the geldanamycin biosynthesis by

	Streptomyces hygroscopicus
h	hour
HCl	hydrochloric acid
HNO_3	nitric acid
HMBC	heteronuclear multiple bond correlation
H_3PO_2	hypophosphorous acid
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
H_2SO_4	sulfuric acid
Hsp90	heat shock protein 90
HSQC	heteronuclear single quantum coherence
Hz	hertz
J	coupling constant
KR	ketoreductase
KS	ketosynthase
L	liter
LDA	lithium diisopropylamide
$LiAlH_4$	lithium aluminium hydride
µ	micro
M	molar
mg	milligram
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MHz	megahertz
min	minute
mL	milliliter
mmol	millimol
M.p.	melting point
MS	mass spectrometry or molecular sieves
MSMS	tandem mass spectrometry
MTPA	α-methoxy-α-(trifluoromethyl)phenylacetyl
m/z	MS: mass/charge
n-BuLi	*n*-butyllithium
NMR	nuclear magnetic resonance spectroscopy
NRPS	non-ribosomal peptide synthetase
PE	petroleum ether
Ph	phenyl
PKS	polyketide synthase
PMB	*para*-methoxybenzyl
PPh_3	triphenylphosphine
ppm	parts per million
PPTS	pyridinium *p*-toluenesulfonate
quant.	quantitative
rpm	rounds per minute
RT	room temperature
SAR	structure-activity relationship
SNAC	*N*-acetylcysteamine
T	temperature
t	time

t_R	retention time
TBAF	tetra-*n*-butylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TBSOTf	*tert*-butyldimethylsilyl trifluoromethanesulfonate
TES	triethylsilyl
TESOTf	triethylsilyl trifluoromethanesulfonate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
UDP	uridine diphosphate
UV	ultraviolet
δ	NMR chemical shift
Δ	heating under refluxing conditions

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Curriculum vitae

Personal data

Name:	Ilona Bułyszko
Date and place of birth:	October 12, 1987; Góra, Poland
Nationality:	Polish
Marital status:	Single

Education

Leibniz Universität Hannover, Hannover, Germany

Doctoral Research Program 2012 – Present
Supervisor: Prof. Dr. Andreas Kirschning
„Studies on Ansamycin Antibiotics: Mutasynthetic Approach Towards New Rifamycin Derivatives and Total Synthesis of Progeldanamycin Derivatives”

Adam Mickiewicz University, Poznań, Poland

M.Sc. in Biotechnology 2010 – 2012
Supervisor: Prof. J. Sadowski
“Elements of Phosphorylation- and Dephosphorylation-Dependent Protein Interaction Networks in *Arabidopsis thaliana*”

M.Sc. in Chemistry: Biological Chemistry 2009 – 2011
Prof. M. Chrzanowska
“Asymmetric Synthesis of 6,7-Dimethoxy-1,2,3,4-Tetrahydroisoquinoline-1-Carboxylic Acid via Petasis Borono-Mannich Reaction”

B.Sc. in Biotechnology 2007 – 2010
Supervisor: Prof. J. Sadowski
“Basics of Protein-Protein Interactions”

B.Sc. in Chemistry: Biological Chemistry 2006 – 2009
Prof. M. Chrzanowska
“Synthesis of 1,2,3,4-Tetrahydroisoquinoline-1-Carboxylic Acid Derivatives (Mammalian Alkaloids) – Review of Synthetic Methods Published from 1970 to 2008”

Adam Mickiewicz High School, Góra, Poland 2003 – 2006

Publications

I. Bułyszko, G. Dräger, A. Klenge, A. Kirschning, *Chem. Eur. J.* **2015,** *21*, 19231−19242.

J. Hermane, I. Bułyszko, S. Eichner, F. Sasse, W. Collisi, A. Poso, E. Schax, J.-G.Walter, T. Scheper, K. Kock, C. Herrmann, P. Aliuos, G. Reuter, C. Zeilinger, A. Kirschning, *ChemBioChem* **2015,** *16*, 302−311.

I. Bułyszko, M. Chrzanowska, A. Grajewska, M. D. Rozwadowska, *Eur. J. Org. Chem.* **2015,** *2*, 383−388.

Scholarships

Dean's Scholarship in Biotechnology	2010 - 2011
Dean's Scholarship in Chemistry	2007 - 2011
Scholarship of The Prime Minister of Poland	2004 - 2005

Conference Meetings and Poster Presentations

19th European Symposium of Organic Chemistry, Lisbon, Portugal	July 2015
15th Leibniz Symposium on Design in Nature and Laboratory, Hannover, Germany	February 2015
14th Belgian Organic Synthesis Symposium, Louvain-la-Neuve, Belgium	July 2014
The Chemistry and Biology of Natural Products Symposium VII, Warwick, UK	June 2013
Materials and Biomaterials, Poznań, Poland, conference organised by Wielkopolska Centre of Advanced Technologies	November 2011
The GlaxoSmithKline Residential Chemistry Training Experience 2011, Stevenage, UK	September 2011
9th All-Polish Symposium of Organic Chemistry, Warsaw; Poland	April 2011